STUDIES IN THE FIELD

OF

BISBENZYLISOQUINOLINE ALKALOIDS

by

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being a Thesis submitted for the degree
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MEMORANDUM

All melting points recorded in this thesis have been corrected and all specific rotations determined, unless otherwise specified, in a two decimetre tube.

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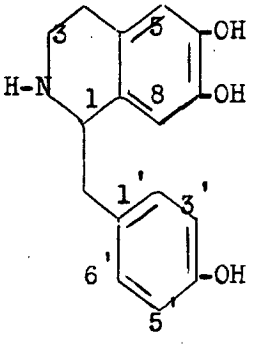
PART 1

INTRODUCTION

The bisbenzyl*iso*quinoline alkaloids are a group of bases occurring in the *Menispermaceae* or closely related families of plants. Structurally they consist of two benzyl*iso*quinoline units joined together by ether linkages ; biogenetically they probably arise by the formation of an ether link between two molecules of the following type by a dehydrogenation mechanism (1, 2, 3, 4).



1.1

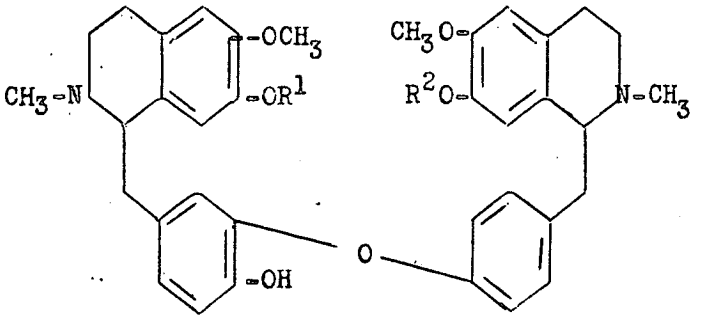
Molecules of type 1.1 also occur in nature. Coclaurine (7, 4'-dihydroxy 6-methoxybenzyl*iso*quinoline) is found in the far eastern plant *Cocculus laurifolius* while *iso*coclaurine (6, 4'-dihydroxy 7-methoxybenzyl*iso*quinoline) has been isolated from " pareira brava " (probably the root of *Chondrodendron platyphyllum*). Thus formula (1.1) is *nor*coclaurine and this provides for the bisbenzyl*iso*-quinoline group the alternate name, biscoclaurine alkaloids.

The number and position of the ether linkages divide the thirty or so alkaloids at present known into several broad classes in which both structural and stereochemical isomerism (asymmetric centre at position 1) are possible while the N-methyl and O-methyl content varies within the group.

The following is a list of the chief representatives of this group of alkaloids and the structures assigned to them at the present time. It is in no way exhaustive and is included purely as a reference table.

TYPE A

This group is characterised by one ether link between the 4' position of one coclaurine unit and the 3' position of another.



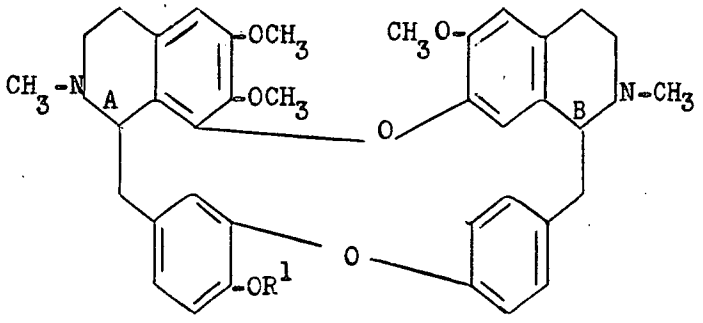
1.11

Dauricine 1.11 (R^1= R^2= CH_3)

Magnoline 1.11 (R^1= R^2= H)

TYPE B

This group is characterised by two ether linkages ; one between the 8 and 7 positions of the two coclaurine units and the other between the 3' position of the first unit and the 4' position of the second.



1.111

Phaeanthine 1.111 (R^1= CH_3)

centres A, B negative

Tetrandrine 1.111 (R^1= CH_3)

centres A, B positive

Isotetrandrine 1.111 (R^1= CH_3)

centre A negative

centre B positive

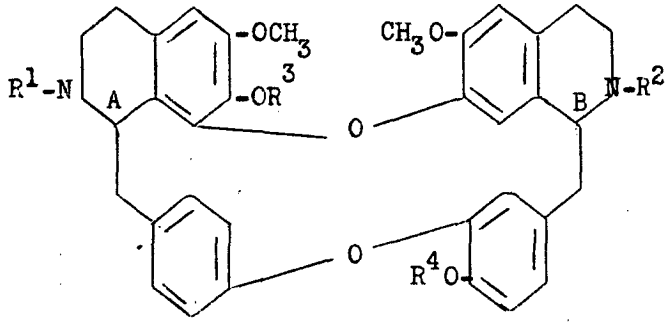
Berbamine 1.111 (R^1= H)

centre A negative

centre B positive

TYPE C

This group again contains two ether linkages ; one between the 8 and 7 positions as before and the other between the 4' position of the first unit and the 3' position of the second.



1.1V

Oxyacanthine 1.1V (R^1= R^2= R^3= CH_3 ; R^4= H)

A probably positive

B probably negative

(see Part 3 of this thesis)

Repandine 1.1V (R^1= R^2= R^3= CH_3 ; R^4= H)

both A and B probably positive

(see Part 3 of this thesis)

Daphnandrine 1.1V (either R^1= H, R^2= CH_3 or

R^1= CH_3, R^2= H ; R^4= CH_3, R^3= H)

A probably positive

B probably negative

Aromoline 1.1V (R^1= R^2= CH_3, R^3= R^4= H)

A probably positive

B probably negative

Daphnoline (Trilobamine)

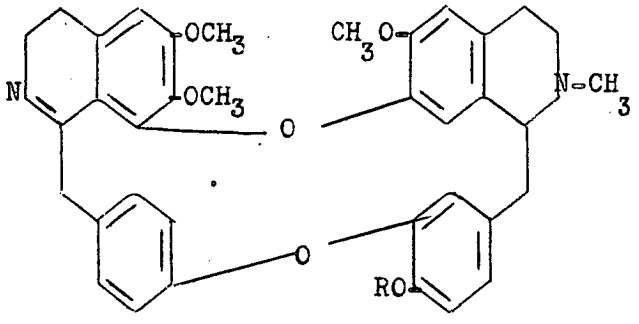
1.1V (either R^1= H, R^2= CH_3 or

R^1= CH_3, R^2= H ; R^3= R^4= H)

A probably positive

B probably negative

Also belonging to this group is a small sub-group which has a double bond in the 1-2 position in one of the isoquinoline rings.



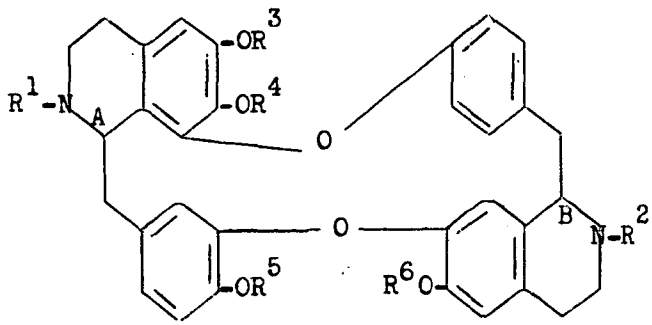
1.V

Epistephanine 1.V (R= CH_3)

Hypoepistephanine 1.V (R= H)

TYPE D

This group also has two ether linkages ; one between the 8 position and the 4' position of two coclaurine units, the other between the 3' position of the first unit and the 7 position of the second.



1.V1

Chondrocurine 1.V1 (R^1= R^2= R^4= R^6= CH_3 ; R^3= R^5= H)

one centre positive

the other negative

Bebeerine 1.V1 (R^1= R^2= R^3= R^6= CH_3 ; R^4= R^5= H)

A and B probably positive

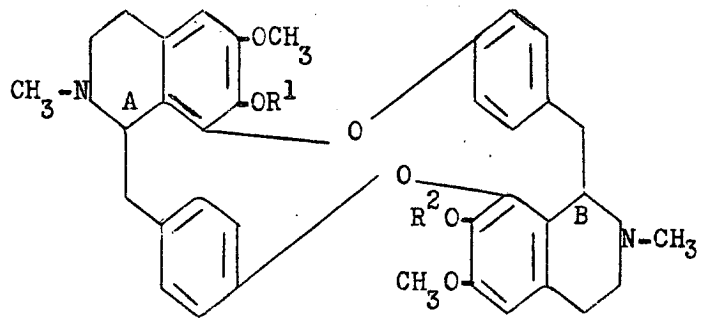
Curine 1.V1 (R^1= R^2= R^3= R^6= CH_3 ; R^4= R^5= H)

A and B probably negative

Chondrofoline 1.V1 (R^1= R^2= H ; either R^3= H, R^4= R^5= R^6= CH_3 or R^6= H, R^3= R^4= R^5= CH_3)

TYPE E

In this group two ether linkages join the two coclaurine residues by their 8 and 4' positions.



1.V11

Isochondrodendrine 1.V11 (R^1= R^2= H)

both A and B centres

probably negative

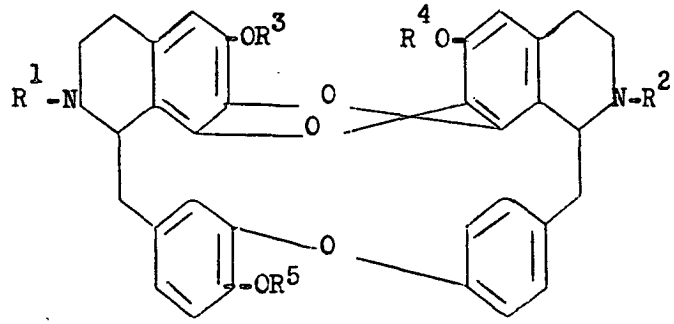
O,O-Dimethylisochondrodendrine (Cycleanine)

1.V11 (R^1= R^2= CH_3)

A and B both negative

TYPE F

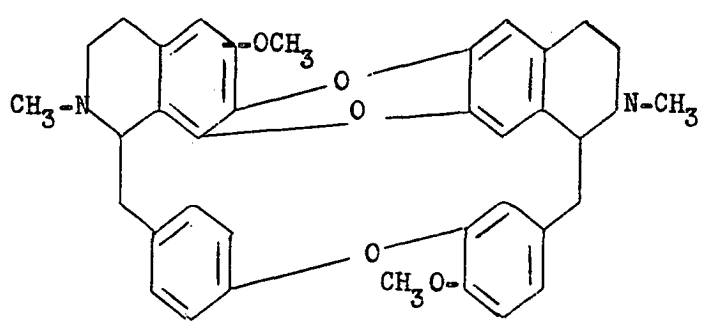
Examples of bisbenzyl<u>iso</u>quinoline bases are known which have three ether linkages.



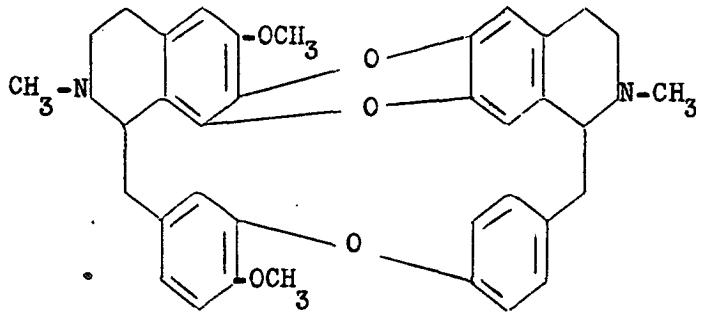
1.V111

Micranthine 1.V111 (R^5= H, of R^1 and R^2 one is H, the other CH_3 ; of R^3 and R^4, one is H, the other CH_3)

Two further examples of bisbenzyl<u>iso</u>quinoline alkaloids having three ether linkages are trilobine and <u>iso</u>trilobine.



1.1X

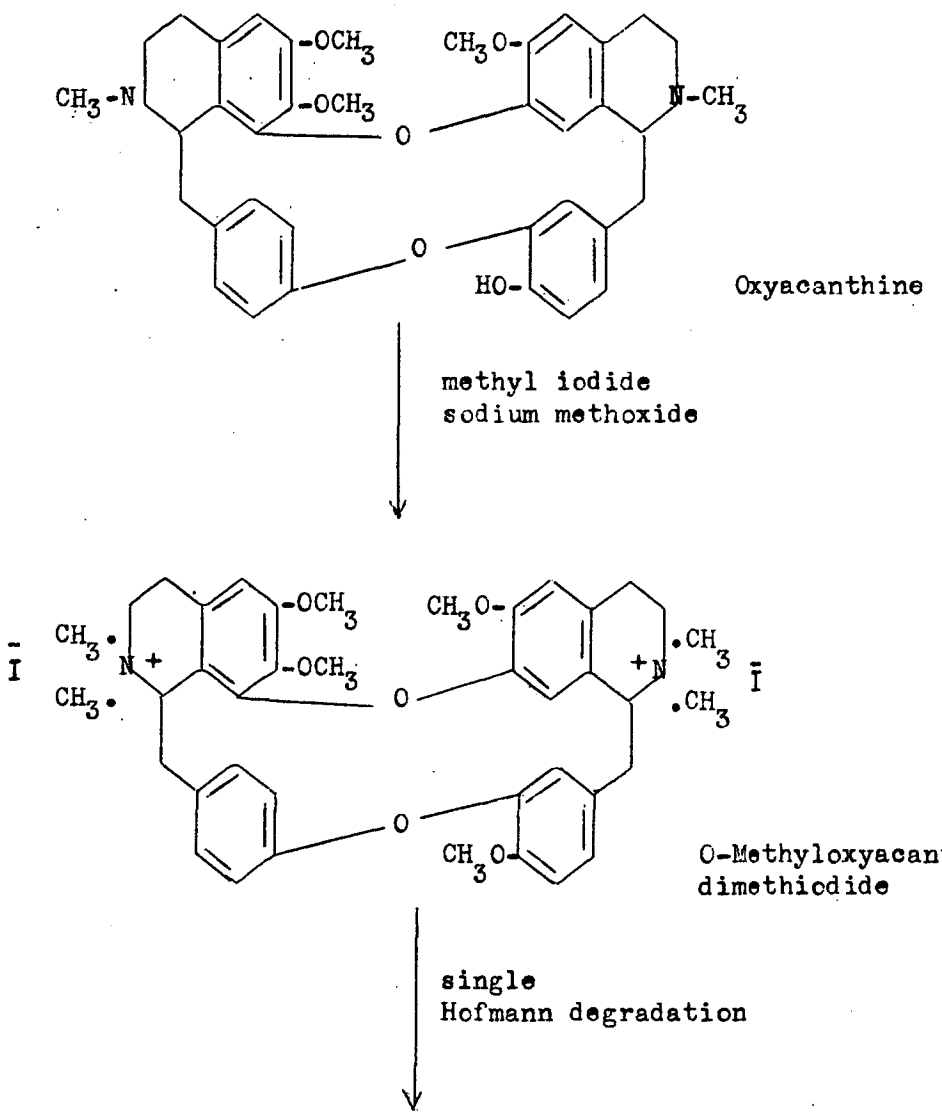


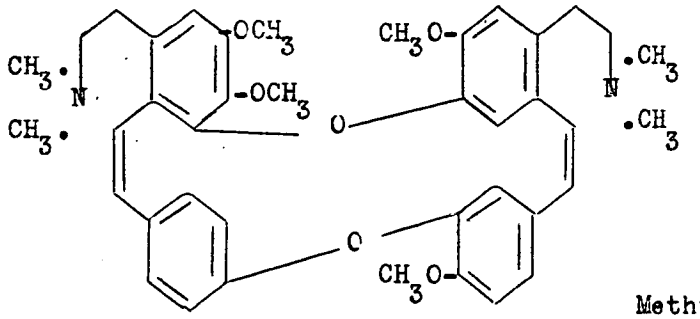
1.X

At the present time it is not possible to assign a definite structure to either of these bases, but if formula 1.1X is trilobine then <u>iso</u>trilobine is 1.X or <u>vice versa</u>.

The classical method of degradation which has been repeatedly followed in this group involved the use of Hofmann degradation followed by permanganate oxidation or ozonolysis of the methine bases so formed. These methods can be best illustrated by selecting two alkaloids (oxyacanthine, bebeerine) and sketching the reactions used to prove their structures.

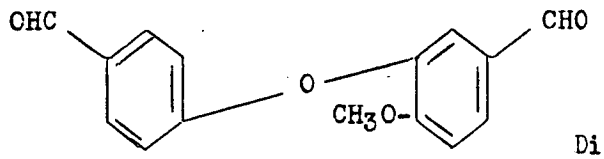
<u>The Degradation of Oxyacanthine</u>





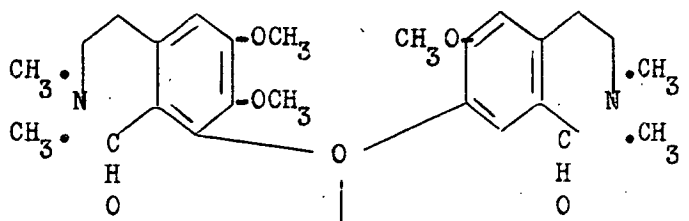
Methine base

ozone



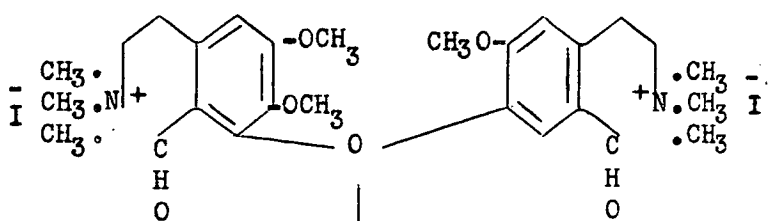
Diphenyl ether dialdehyde

AND



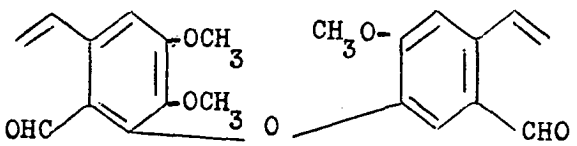
Amino-aldehyde

methyl iodide



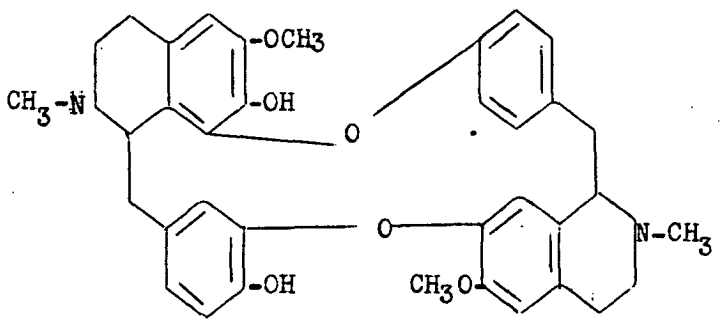
Amino-aldehyde dimethiodide

Hofmann degradation



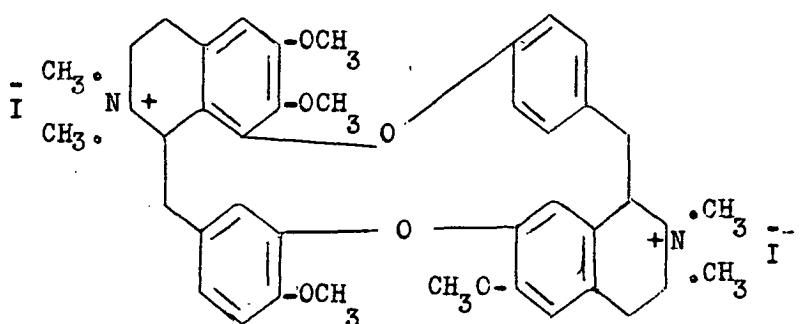
Divinyl diphenyl ether dialdehyde

The Degradation of Bebeerine



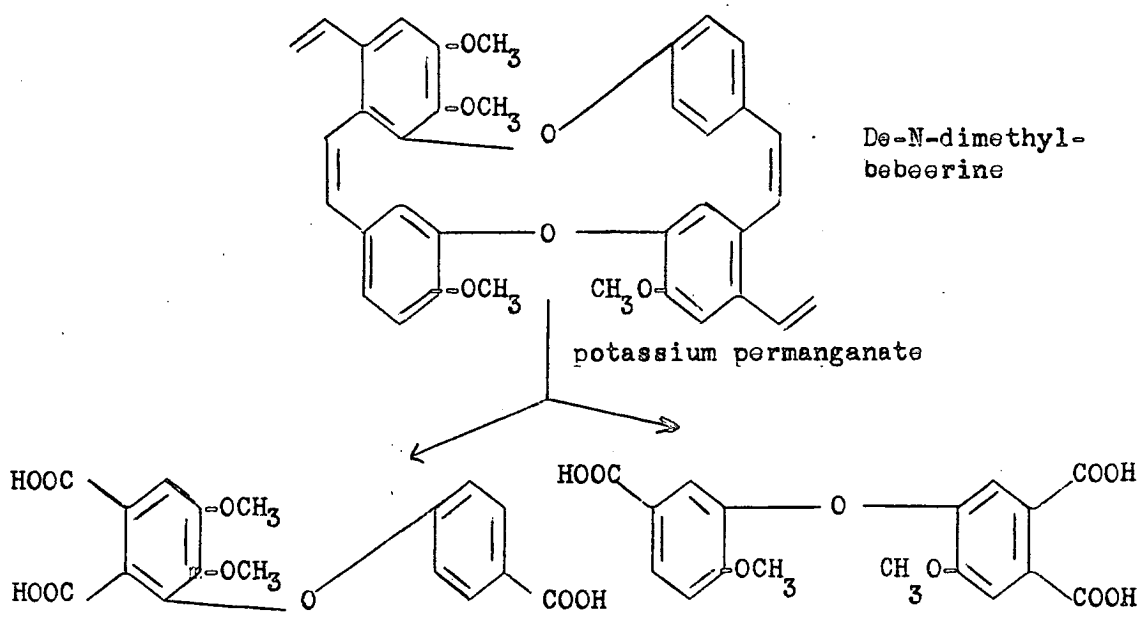
Bebeerine

methyl iodide
potassium hydroxide



O,O-Dimethyl-bebeerine dimethiodide

double
Hofmann degradation



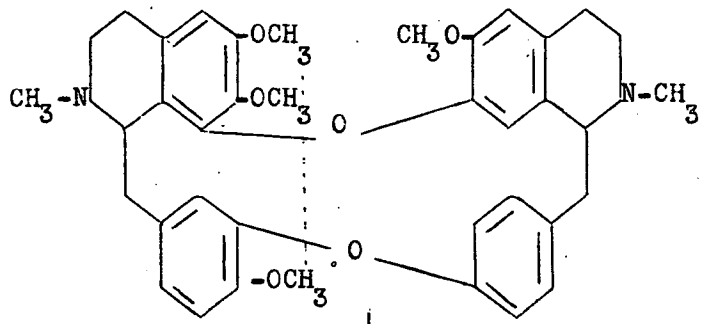
The final products in each of the above degradations were either compared directly with known compounds or synthesised to confirm their structure.

However while these methods of degradation gave a general picture of the molecular structure, in many cases finer points such as the position of methylimino and phenolic groups were left unsolved. Still more serious structural ambiguities remained in some cases, the most important being the lack of distinction between the oxyacanthine and tetrandrine type of molecule ; moreover little insight was gained into the nature of the asymmetric centres.

Recently a new type of degradation has been devised by Tomita and others (5-10). In their process the alkaloids are reduced with sodium in liquid ammonia which results in the fission of the ether linkages. The important feature of this method is that in all cases so far examined the fission products were always of the coclaurine type (formula 1.1) and the other benzyl*iso*-quinoline units which could be formed from such a fission, if present at all, were in negligible amounts. Thus it appears that the reductive fission represents a reversal of what is no doubt the biogenetic method of synthesis of these alkaloids from two coclaurine units.

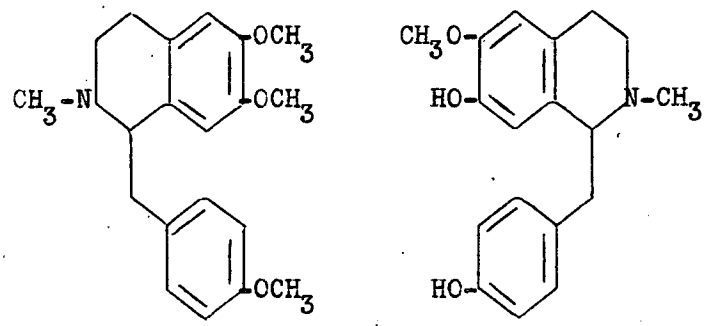
It is interesting to note that quite recently Kidd and Walker (11, 12) have found that the fission products obtainable from this reduction with sodium in liquid ammonia depend on the solvent employed. The Japanese workers used benzene-toluene mixtures as solvents for their alkaloids and obtained only coclaurine fission products. Kidd and Walker confirmed the Japanese results with benzene and toluene but obtained a very complex mixture of bases when dioxan was used as the solvent.

Tomita and his co-workers were interested in this reductive fission chiefly as a means of settling the



1.111

sodium
liquid ammonia

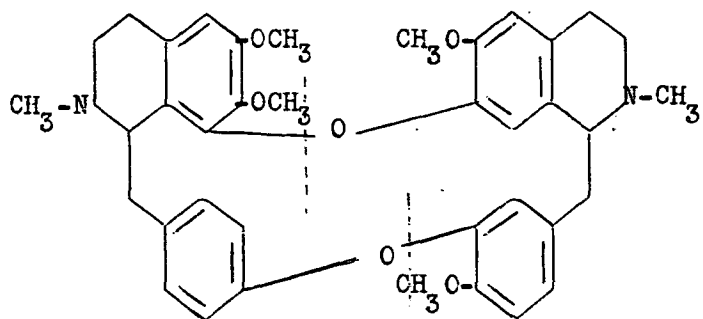


1.X11 1.X1

difference between the tetrandrine (type B) group and the oxyacanthine (type C) group. In their investigations they found that <u>iso</u>tetrandrine (5, 6) when cleaved with sodium in liquid ammonia yielded two bases almost quantitatively. One of these was phenolic and was shown to be (+)N-methylcoclaurine (1.X1) while the other was non-phenolic. This latter base was proved identical with (-)O-methylarmepavine (1.X11). Thus the fission

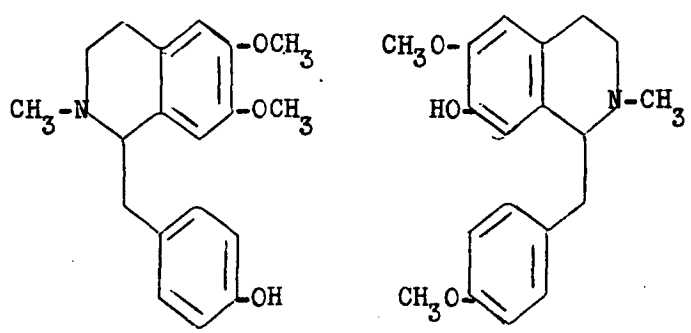
must have occurred by the plan shown above and iso-tetrandrine can be represented by formula 1.111.

Oxyacanthine when methylated and subjected (9) to this reductive cleavage yielded two phenolic bases which proved to be (+)armepavine (1.X111) and a base which must be (-)N,O-dimethylcoclaurine (1.X1V). Thus the fission of O-methyloxyacanthine follows the course shown below and establishes its structure as (1.1V).



1.1V

sodium-liquid ammonia



1.X111 1.X1V

At the same time the Japanese workers were able by an examination of the rotations of the two coclaurine halves to gain an insight into the stereochemistry of the parent alkaloids. Since that time they have extended their work by way of cleavage experiments on other alkaloids with a view to examining their stereochemistry. Their results are reported in Table 1.1.

TABLE 1.1

Alkaloid	$[\alpha]_D$	Fission Products	Reference
Isotetrandrine	+146	(-)O-methylarmepavine (+)N-methylcoclaurine	5, 6
Tetrandrine	+270	(+)O-methylarmepavine (+)N-methylcoclaurine	7
O,O-Dimethyliso-chondrodendrine	-15	(-)armepavine only one fission product	8
Oxyacanthine	+278	(+)armepavine (-)N,O-dimethylcoclaurine	9
Repandine	-106	(+)armepavine (+)N,O-dimethylcoclaurine	10

Thus it could be seen that isotetrandrine and oxyacanthine each contained a d and an l asymmetric centre ; tetrandrine and repandine had both their centres of the

d form while in 0,0-dimethylisochondrodendrine both centres were of the l type. In the main these results were in accord with those generally expected from an examination of the sign and magnitude of the rotations of these bases. The notable exceptions were oxyacanthine and repandine for which a (++) and (+-) formulation respectively had been expected. The Japanese explained this anomoly by the occurrence of a Walden Inversion in these bases during the reductive fission. Support for this was derived from the fact reported by von Bruchhausen (13) that oxyacanthine can be converted into repandine by hydrochloric acid in ethanol. This process would require a Walden Inversion and if correct would indicate the instability of one asymmetric centre in oxyacanthine. A discussion of these results will be deferred until later in this thesis (Part 3).

It is the aim of this thesis to extend the cleavage experiments of the Japanese workers to other bisbenzylisoquinoline alkaloids to aid in the elucidation of their structure and stereochemistry.

The extraction methods followed in the isolation of these alkaloids have as far as possible been modern techniques such as chromatography and counter-current extraction. As a result new minor bases have been found in previously investigated species. The isolation of

these minor bases strongly indicates the need for re-examination of well known alkaloid-bearing species which have been the subject of investigation by older methods of extraction, separation and purification.

PART 2

ATHEROSPERMINE

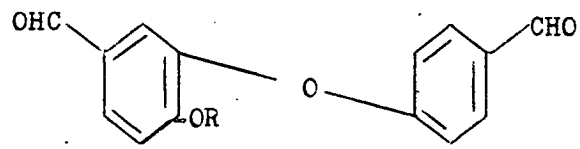
THE MAJOR ALKALOID OF ATHEROSPERMA MOSCHATUM

Atherosperma moschatum (family Monimiaceae) is a tree of average dimensions, endemic in Australia and restricted to the South Eastern corner, occurring only in Tasmania, Victoria and southern New South Wales. Botanically it is closely related to the Daphnandra species of Queensland and New South Wales which have yielded a group of well investigated alkaloids of the bisbenzylisoquinoline type. Pharmacologically, Sassafras, which is the local name for Atherosperma, has been employed as a beverage under the titles of " Sassafras Beer " or " Sassafras Tea ". The older settlers claimed that these drinks had marked curative properties for many ills. The presence of alkaloids in the bark of this species was reported as early as 1861 by Zeyer (14) who was probably the first person to isolate an alkaloid from an Australian plant. However as a result of his work only analytical figures (C_{30} H_{40} O_5 N_2) and a melting point (128°C) were reported for this base which was named atherospermine. Interest then lapsed in this compound until recently when W. D. Crow (15) of C.S.I.R.O. Melbourne began an investigation of the alkaloidal content of Sassafras in which he found the main base occurring

to the extent of 1.8% together with small amounts of other bases. This major base adhering to Zeyer's nomenclature was called atherospermine. Dr. Crow fully characterised atherospermine and established its molecular formula as (C_{37} H_{40} O_6 N_2) but was unable to carry on with degradative work and the problem together with a considerable quantity of purified base was handed on to the present author through the kindness of Drs. Crow and Price.

The work of Crow in establishing the above molecular formula for atherospermine makes this base isomeric with oxyacanthine, berbamine and other bisbenzyl*iso*quinoline alkaloids. The presence of three methoxyl and two methylimino groups was confirmed and it was demonstrated that atherospermine had weak phenolic properties.

Methylation of the base with methyl iodide in methanolic sodium methoxide yielded O-methylatherospermine dimethiodide ; Hofmann degradation of this product gave a mixture of methines which were difficult to separate. When the mixture was ozonised a diphenyl ether dialdehyde (2.1, R= CH_3) was formed which by direct comparison with an authentic specimen prepared analogously from repandine (21) was shown to be 2-methoxydiphenyl ether -5:4'-dialdehyde. The isolation of this compound confirmed the view that atherospermine was a bisbenzyl*iso*-



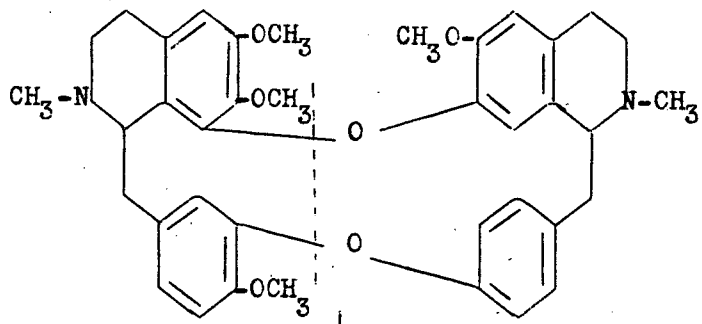
2.1

quinoline alkaloid and also indicated that the benzyl residues were ether-linked to each other as in oxyacanthine, tetrandrine etc. and not to isoquinoline residues as in the Chondrodendron group of alkaloids. However this type of degradation as pointed out in the introduction is ambiguous and does not distinguish between alkaloids of the oxyacanthine and tetrandrine type both of which would give the same products on Hofmann degradation followed by ozonolysis.

In order to distinguish between these two types, O-methylatherospermine was degraded by fission with sodium in liquid ammonia. An initial difficulty was found in methylating the very weakly phenolic hydroxyl group of atherospermine with diazomethane, and an alternative method was tried which involved protection of the amino groups from quaternisation by conversion to the N-oxide, followed by methylation of the phenolic group with dimethyl sulphate and sodium hydroxide. Reduction of the di-N-oxide then gave the desired O-methylatherospermine.

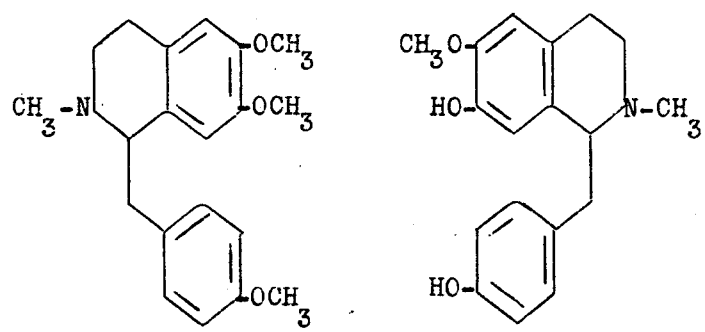
Subsequently however it was found possible to methylate satisfactorily with diazomethane by modifying the conditions and using reaction periods of 2-3 weeks.

As a preliminary trial of the fission method, and in order to secure comparison samples of known constitution the alkaloids phaeanthine (2.11) and O,O-dimethyl<u>iso</u>-chondrodendrine (cycleanine) (2.V) were cleaved with sodium in liquid ammonia. Although the base phaeanthine



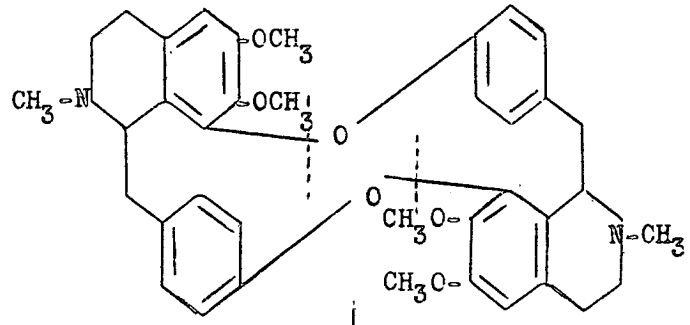
2.11

sodium
liquid ammonia



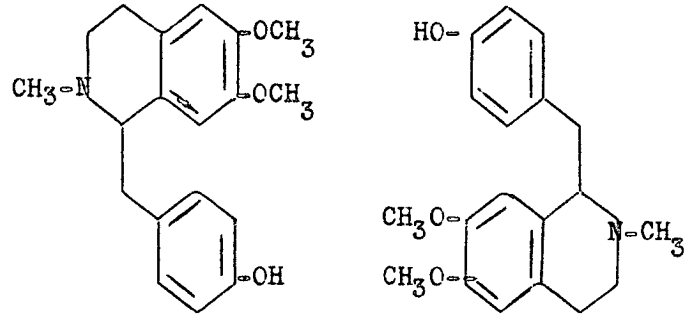
2.111

2.1V



2.V

sodium
liquid ammonia



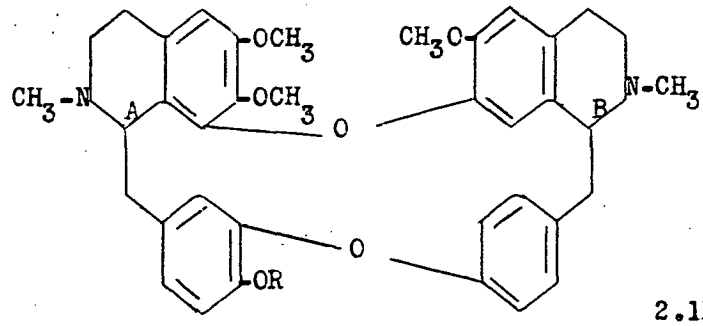
2.VI 2.VI

had not been cleaved previously by this method it was known from the Japanese investigation of the enantiamorphic alkaloid tetrandrine (7) that the products of fission would be (-)O-methylarmepavine (2.111) and (-)N-methylcoclaurine (2.1V). More recently Kidd and Walker (11, 12) have also examined phaeanthine by using this reductive fission and report the same products as

indicated above. O,O-Dimethyl*iso*chondrodendrine (2.V) had already been cleaved by Tomita, Fujita and Murai (8) who showed that (-)armepavine (2.V1) was the only product. These substances isolated as described by the Japanese workers, corresponded in properties to the expected coclaurine derivatives.

Fission of O-methylatherospermine with sodium in liquid ammonia gave two products, one phenolic and one non-phenolic and these were identified as (-)O-methyl-armepavine (2.V11, R= CH_3) and (+)N-methylcoclaurine (2.V111), two simple benzyl*iso*quinoline bases. These were the same products obtained by the Japanese workers from the fission of the alkaloid *iso*tetrandrine (5, 6) and this coupled with the previous isolation of 2-methoxydiphenyl ether -5:4'-dialdehyde as a degradation product made it virtually certain that O-methyl-atherospermine was identical with *iso*tetrandrine. Direct comparison with an authentic specimen of *iso*tetrandrine confirmed this identity. Hence O-methylatherospermine can be represented by formula (2.11 or 2.1X, R= CH_3) where the asymmetric centres A and B have different configurations.

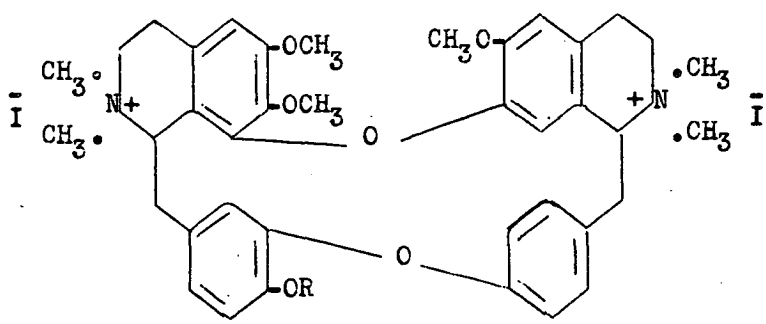
The next question to be decided was the position of the hydroxyl group in the original atherospermine. When

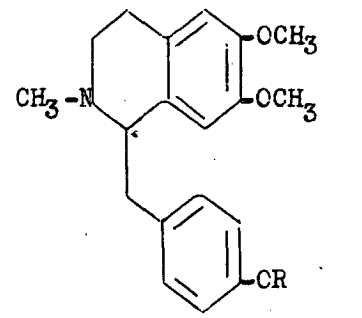


2.1X

ethyl or
methyl iodide

sodium
liquid ammonia

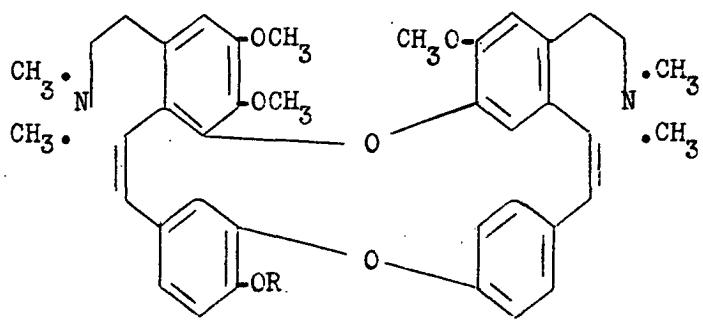


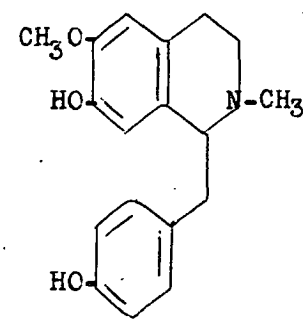


2.V11

AND

Hofmann
degradation

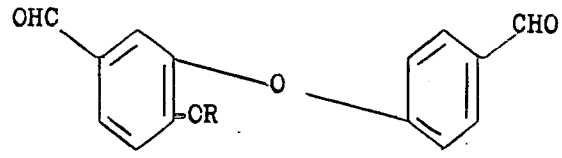




2.V111

Optically
inactive
methine

ozone



2.1

atherospermine was ethylated and submitted to Hofmann degradation followed by ozonolysis, the ethoxydiphenyl ether dialdehyde (2.1, R= C_2H_5) corresponding to the previous methoxyl compound was isolated, although in rather poor yield. This indicated that the hydroxyl group in atherospermine was in the benzyl group and this fact was confirmed by repeating the sodium in liquid ammonia fission on O-ethylatherospermine (2.1X, R= C_2H_5). The products obtained were (+)N-methylcoclaurine (2.V111) and O-ethylarmepavine (2.V11, R= C_2H_5). The identity of this last compound was established by comparing directly its methiodide with the methiodide prepared from the ethylation of (-)armepavine derived from the fission of (-)O,O-dimethyl<u>iso</u>chondrodendrine. This series of experiments showed that atherospermine could be represented by formula (2.1X, R= H).

A structure of this type had been assigned to berbamine (16), an alkaloid occurring as a minor constituent in several berberidaceous plants. Like

atherospermine it had a phenolic group and when methylated it yielded isotetrandrine ; however the position of the hydroxyl group had never been fixed and could be in one of the other three positions in atherospermine occupied by methoxyl groups. In order to see whether the two alkaloids were identical, a small sample of berbamine was obtained from Professor Tomita in Japan for a direct comparison. However many bases in this series, particularly those containing phenolic groups, retain solvent of crystallisation to a remarkable extent, and largely as a result of this the melting point of berbamine is variously reported in the literature as 122-125°, 156°, 172° and 197-210° (30) according to the methods of crystallisation and drying and a similar spread of m.ps. was found with atherospermine. The method of mixed m.ps. was thus unsatisfactory, and a comparison of Debye-Scherrer diagrams and infra-red spectra was also indecisive. In order to make certain of the identity, a quantity of berbamine was isolated from barberry (see next section), from which it had been first reported, and ethylated. The O-ethyl compounds melt sharply and the m.p. of a mixture was not depressed ; moreover O-ethylberbamine gave the same products on degradation with sodium in liquid ammonia as

atherospermine. Thus it is established that atherospermine and berbamine are identical and the position assigned without proof in the earlier literature to the hydroxyl group of the latter is correct.

From a pharmacological view point Sassafras has interesting possibilities. It has been mentioned above that the early Australian pioneers attributed remarkable cures to potents incorporating extracts of this bark. It was probably these reported therapeutic properties that originally interested Zeyer (14) in Atherosperma moschatum and led Stockman (17) at Edinburgh to investigate the species from a pharmaceutical point of view. However the latter's findings were not encouraging and interest was lost in this plant. The following is an extract of Stockman's paper.

" It seems therefore certain, that neither the volatile oil nor any other constituent of the bark of Atherosperma moschatum is particularly active or poisonous, and further that the volatile oil has a close resemblance in physiological action to other volatile oils. Regarding its use as a diaphoretic, expectorant,and alterative, there is little doubt that it is simply similar to the many other essential oils or plants containing them which are used in medicine for similar purposes ". [The essential oil of Atherosperma moschatum

was later investigated by Scott (107)].

However Marsh (18, 19) recently has shown that berbamine dimethiodide has a curariform action about equal to that of tubocurarine chloride the standard substance used clinically as a muscle relaxant, and at the same time it is slightly superior to tubocurarine chloride in having fewer undesirable side effects. Up to the present berbamine has been a rather rare alkaloid and its presence in quantity in the readily available Sassafras bark, from which it can easily be isolated and its ready quaternisation in excellent yields either to the dimethiodide or dimethochloride may provide an alternative to tubocurarine chloride as a curariform agent. At the present time both these derivatives are undergoing clinical tests in London through the co-operation of the Wellcome Foundation.

EXPERIMENTAL

Extraction of Atherospermine. Sundried Sassafras bark (3.0 Kg.) was milled and extracted by stirring overnight with aqueous tartaric acid (15 litres ; ¼%). By centrifugation the extract was freed from the bark which was subjected again to a fresh tartaric acid solution. This process was repeated until the bark gave only weak Meyer's tests. At this stage the extracts were combined (120 litres) and evaporated under reduced pressure to a more convenient volume (10 litres), when the bases were precipitated by the addition of sodium carbonate. The crude alkaloid was filtered, dried in a vacuum desiccator and extracted with chloroform in a modified Soxhlet type (20) apparatus under reduced pressure so that the temperature was at no time greater than 30°. The chloroform solution (5 litres) was reduced by two thirds in volume, extracted with sodium hydroxide (5 x 2%) to remove phenolic bases, washed with water and evaporated in vacuo to dryness. The resultant gummy residue was extracted repeatedly with boiling benzene, filtered hot and allowed to cool. A crystalline benzene adduct of atherospermine (51 g.) settled out.

Atherospermine. A specimen of the alkaloid isolated as

described above was purified by recrystallisation from aqueous methanol and benzene and formed colourless needles m.p. 128-135°d., $[\alpha]_D^{20}$= +114 (c, 0.5 in $CHCl_3$). It gave no coloration with ferric chloride but produced a pink colour when allowed to stand with Millon's reagent for several hours. When excess alkali (1% NaOH) was added to a solution of the base in acid (1% HCl) a precipitate was formed which redissolved to a clear solution ; carbonation of this produced a white precipitate which after recrystallisation from benzene possessed identical properties with those of the original base.

Found

C,75.0 H,6.7 N,4.2 NCH_3,7.3 CH_3O,13.8%

Calc. for $C_{37}H_{40}O_6N_2.C_6H_6$

C,75.2 H,6.8 N,4.1 $2NCH_3$,8.5 $3CH_3O$,13.6%

<u>O-Methylatherospermine dimethiodide</u>. Atherospermine (5 g.) was dissolved in dry methanol (250 c.c.) and methyl iodide (7.5 c.c.) was added followed by methanolic sodium methoxide (0.32 g. in 5 c.c.). During a period of 36 hours the mixture was refluxed and further similar additions of methanolic sodium methoxide were made at six hourly intervals. The solvent was then removed <u>in vacuo</u>

and a solution of the residue in hot water was boiled with a little copper powder and activated charcoal for 10 minutes, filtered hot and allowed to cool. The amorphous solid which separated was collected and crystallised from methanol yielding fine needles of O-methylatherospermine dimethiodide (6 g.) which decomposed without melting at 220-250° and had $[\alpha]_D^{20}$= -63.6 (c, 0.5 in H_2O) or -33.8 (c, 0.3 in CH_3OH). An authentic sample of isotetrandrine dimethiodide decomposed similarly on heating and had $[\alpha]_D^{20}$= -30 (c, 0.3 in CH_3OH).

Found

C,49.5 H,5.9 I,25.5 CH_3O,12.8%

Calc. for $C_{40}H_{48}O_6N_2I_2.4H_2O$

C,49.1 H,5.8 I,25.9 $4CH_3O$,12.7%

O-Methylatherospermine Methines. O-Methylatherospermine dimethiodide (5 g.) was dissolved in water (1.5 litres) and shaken with freshly prepared silver oxide until the solution was free from iodide ions. After filtration and concentration in vacuo the solution (100 c.c.) was treated with aqueous potassium hydroxide (50 c.c. of 50%) and heated on a steam bath for 2 hours. The solution and the brown resin which separated from it were extracted with chloroform and the process of heating and extraction of the aqueous solution was repeated until no more methine

was formed. The chloroform extracts were combined, washed with water, dried with sodium sulphate and evaporated.

2-Methoxydiphenyl ether -5:4'-dialdehyde. The mixture of methine bases (2 g.) was dissolved in dilute sulphuric acid (20 c.c. of 5%), the solution cooled in a freezing mixture and a stream of ozone passed through it for 5 minutes. The solution and the solid which separated from it were extracted with ether and the process of ozonolysis and extraction was repeated until no more solid was precipitated. The ethereal extracts were combined, washed with sodium carbonate solution and water and finally dried with sodium sulphate. After removal of the solvent the residue was crystallised from light petroleum (40-60°), yielding colourless needles m.p. 74-6°. A mixed m.p. determination with a specimen of 2-methoxydiphenyl ether -5:4'-dialdehyde (m.p. 77°) prepared by Bick and Todd (21) by a similar series of reactions from repandine showed no depression.

O-Methyl-de-N-atherospermine. A mixture of O-methyl-atherospermine methines (1 g.) was dissolved in methanol (25 c.c.) and boiled under reflux during 15 minutes with methyl iodide (2 c.c.). The solution was evaporated to dryness under reduced pressure and dissolved in water

(1 litre). After the free iodide ions had been removed with silver oxide the solution was heated on a water bath with potassium hydroxide (50 c.c. ; 50%) when trimethylamine was evolved and a resin slowly separated. The latter was extracted from the cooled solution with chloroform and the heating and extraction repeated until no further resin settled out. The chloroform solutions were combined, washed with water, dried over sodium sulphate and evaporated under reduced pressure. The residue when crystallised and recrystallised from a chloroform-glacial acetic acid mixture had m.p. 212°.

Found

C,75.9 H,5.8 CH_3O,21.4%

Calc. for $C_{36}H_{32}O_6 \cdot \frac{1}{2}H_2O$

C,75.8 H,5.8 $4CH_3O$,21.7%

O-Ethylatherospermine dimethiodide. Atherospermine (5 g.) was dissolved in methanol (200 c.c.), the solution was refluxed with methyl iodide (5 c.c.) for 15 minutes and then evaporated to dryness in vacuo. The solid dimethiodide was dissolved in hot ethanol (500 c.c.) and the solution treated with ethyl iodide (12.5 c.c.)

followed by ethanolic sodium ethoxide (0.9 g. in 10 c.c.). The mixture was refluxed for six hours whereupon a similar quantity of sodium ethoxide solution was added, and the process of refluxing and addition of sodium ethoxide was continued until the solution had been refluxed 36 hours in all. The solvent was then replaced by water and the solution was boiled with copper powder and activated charcoal for 10 minutes, filtered hot and allowed to cool. O-Ethylatherospermine dimethiodide (5 g.) separated as a white amorphous solid which could not be crystallised.

2-Ethoxydiphenyl ether -5:4'-dialdehyde. O-Ethylatherospermine dimethiodide (5 g.) was submitted to Hofmann degradation as described above, and the resultant mixture of methines ozonised. The resin which settled out was dissolved in ether and the solution was washed with sodium carbonate solution, then with water, dried over sodium sulphate and evaporated. The residue, crystallised from light petroleum (40-60°), gave needles (0.2 g.) m.p. 59°.

Found

C,70.9 H,5.5 C_2H_5O,16.8%

Calc. for $C_{16}H_{14}O_4$

C,71.1 H,5.2 $1C_2H_5O$,16.7%

A mixed m.p. determination with 2-methoxydiphenyl ether -5:4'-dialdehyde showed a marked depression. Bick and Todd (21) report 59° as the m.p. for the above dialdehyde prepared analogously from repandine.

O-Methylatherospermine (a) Via atherospermine di-N-oxide. Atherospermine (5 g.) was dissolved in acetone (50 c.c.) and the solution after addition of hydrogen peroxide (1 c.c. of 30%) was set aside for 10 days during which time a similar addition of hydrogen peroxide was made every 2 days. From the solution, evaporated almost to dryness in vacuo, the N-oxide was isolated as a white hygroscopic mass, readily soluble in water and insoluble in organic solvents. It could not be obtained in a satisfactory state of purity for analysis but its identity was checked by its reduction to atherospermine. For this purpose atherospermine di-N-oxide (1 g.) was dissolved in aqueous ethanol (1:2) and reduced overnight with zinc and hydrochloric acid (36%). To the solution ammonium chloride and aqueous ammonia (S.G. 0.88) were added and the precipitated base was extracted with

chloroform. The residue from the dried and evaporated solution, crystallised from benzene, yielded atherospermine (0.8 g.).

The residual di-N-oxide (4 g.) was dissolved in water (100c.c.) containing sodium hydroxide (1 g.) and freshly distilled dimethyl sulphate (1 c.c.). The mixture was stirred overnight at room temperature after which the excess dimethyl sulphate was removed by repeated extraction with ether. The solution of O-methylatherospermine di-N-oxide was then reduced as described above with zinc and hydrochloric acid. The resultant O-methylatherospermine in benzene solution was purified by chromatography on alumina and after recrystallisation from acetone gave prisms (0.9 g.) with m.p. 181° undepressed on admixture with an authentic specimen of <u>iso</u>tetrandrine.

Found

C,72.9 H,6.7 N,4.6 CH_3O,19.7%

Calc. for $C_{38}H_{42}O_6N_2$

C,73.3 H,6.8 N,4.5 $4CH_3O$,19.9%

The base had $[\alpha]_D^{20}$= +144 (c, 0.5 in $CHCl_3$) compared with $[\alpha]_D^{19}$= +146 for <u>iso</u>tetrandrine m.p. 181-2° as reported

by Kondo and Keimatsu (23).

(b) With diazomethane. To a methanolic solution of atherospermine (1 g. in 30 c.c.) ethereal diazomethane (from 2 g. of nitrosomethylurea) was added and the mixture allowed to stand for two days. After a further two such additions of diazomethane had been made at four day intervals crystals of O-methylatherospermine appeared in the mixture and further quantities of the base were obtained by concentration of the mother liquor. After recrystallisation from acetone, O-methylatherospermine (0.9 g.) had m.p. 181-2° not depressed on addition of isotetrandrine.

Fission of Phaeanthine. Phaeanthine (1 g.) was dissolved in benzene-toluene solution (15 c.c. of each) and liquid ammonia (500 c.c.) was added. To the solution small pieces of clean metallic sodium (2 g. in all) were added gradually with vigorous stirring until the blue colour of the solution persisted for about an hour ; then the mixture was allowed to stand overnight to permit the evaporation of the ammonia. Water and ether were added to the residue and the ethereal phase, separated from the aqueous phase, was washed twice with 2% sodium hydroxide solution. The alkaline washings were combined with the aqueous phase and reserved for the recovery of the phenolic fission product. The ether-benzene-toluene

solution containing the non-phenolic product was exhaustively extracted with hydrochloric acid (5%) and the extract made alkaline with aqueous sodium hydroxide (2%). The non-phenolic base thus precipitated was re-extracted with ether and the ethereal solution was dried over sodium sulphate and evaporated. The residue, dissolved in benzene, was purified by chromatography on alumina and yielded a light oil which was warmed with methanolic methyl iodide (0.1 g. in 2 c.c.). The methiodide of the non-phenolic base separated as needles (0.4 g.) and after being purified by recrystallisation from methanol sintered at 128° and melted at 135° and had $[\alpha]_D^{20} = -118.1$ (c, 0.5 in CH_3OH).

Found

C,51.4 H,6.6%

Calc. for $C_{20}H_{25}O_3N.CH_3I.H_2O$

C,51.7 H,6.2%

Tomita, Fujita and Murai (5, 6) report m.p. 135° for O-methylarmepavine methiodide.

The phenolic base was recovered from the aqueous alkaline solution by addition of ammonium chloride and extraction with ether. The ethereal solution was dried (sodium sulphate) and evaporated to a yellow resin which was difficult to crystallise. The resin was dissolved in

methanol and methylated with an ethereal solution of diazomethane (from nitrosomethylurea). The mixture was allowed to stand for a day, then a further addition of ethereal diazomethane was made. After two days the ether, excess diazomethane and methanol were removed in vacuo and the residue, dissolved in benzene was chromatographed on alumina. A yellow oil was obtained which was converted to its crystalline methiodide by treatment with excess methanolic methyl iodide. Purified by recrystallisation from methanol, the methiodide had $[\alpha]_D^{20} = -114.1$ (c, 0.5 in CH_3OH) and m.p. 136°. No depression of m.p. was observed when a mixed m.p. determination of this methiodide with the one isolated in the non-phenolic fraction was carried out.

Found

C,50.9 H,6.4%

Calc. for $C_{20}H_{25}O_3N.CH_3I.1\frac{1}{2}H_2O$

C,50.8 H,6.3%

Fission of O,O-Dimethylisochondrodendrine (Cycleanine). O,O-Dimethylisochondrodendrine (0.7 g.) was dissolved in benzene-toluene mixture (40 c.c.) and cleaved with sodium (1.5 g.) in liquid ammonia (500 c.c.). The non-phenolic base (0.2 g.) was separated from the phenolic base as

described above and crystallised from acetone. From this solvent fine needles were formed m.p. 270° undepressed by the original O,O-dimethylisochondrodendrine.

The phenolic base (0.5 g.) was dissolved in the minimum amount of ethanol (3 c.c.) and a saturated solution of oxalic acid in ethanol added (2 c.c.). After some hours a crystalline precipitate settled out which after repeated crystallisations from ethanol had m.p. 211°. Armepavine oxalate has m.p. 211-12° (8). The purified oxalate was dissolved in water with warming and the base liberated by the addition of ammonia. Extraction with ether, followed by drying and removal of the solvent left a resin which crystallised from a mixture of acetone and ether. These crystals had a m.p. 143° which were in agreement with Tomita's (10) m.p. of 145° for armepavine.

O-Ethylarmepavine. (-)Armepavine (0.2 g.) was dissolved in methanol (10c.c.) and ethylated with ethereal diazoethane (from 1 g. of ethylnitrosourea). Each day a further similar amount of ethereal diazoethane was added until the fourth day when the solvent was removed in vacuo and the residue purified by chromatography in benzene on an alumina column. (-)O-Ethylarmepavine proved difficult to crystallise but when a methanol

solution of the base was warmed with excess methyl iodide a crystalline deposit of (-)O-ethylarmepavine methiodide was obtained on cooling, m.p. 195°, $[\alpha]_D^{18}$= -81 (c, 0.3 in CH_3OH).

Found

C,54.2 H,6.2%

Calc. for $C_{21}H_{27}O_3N.CH_3I.\frac{1}{2}H_2O$

C,54.2 H,6.3%

Fission of O-Methylatherospermine with Sodium in Liquid Ammonia. O-Methylatherospermine (0.8 g.) was dissolved in a toluene-benzene mixture (2:1) and to this solution, liquid ammonia (400 c.c.) was added, followed by clean pieces of sodium metal (2 g. in all) as previously described. The non-phenolic and phenolic fractions were separated and purified as described for phaeanthine. The non-phenolic base was warmed with methanolic methyl iodide to convert it into its methiodide which when recrystallised from methanol gave needles (0.4 g.) with m.p. 135° (sintering 128°) and $[\alpha]_D^{20}$= -118.5 (c, 0.5 in CH_3OH). No m.p. depression was noted when the methiodide was mixed with an authentic specimen of (-)O-methylarmepavine methiodide (obtained from the cleavage of phaeanthine previously described).

Found

C,51.4 H,6.6%

Calc. for $C_{20}H_{25}O_3N.CH_3I.H_2O$

C,51.7 H,6.2%

The phenolic base when treated with diazomethane gave the O-methyl ether which was purified by chromatography and identified as usual as its methiodide m.p. 134°, $[\alpha]_D^{18}$= +120.1 (c, 0.3 in CH_3OH). A marked depression of m.p. resulted from admixture with a specimen of (-)O-methylarmepavine. The correspondence of constants including the equal and opposite rotation indicated that this methiodide was the optical isomer of (-)O-methyl-armepavine methiodide.

Found

C,51.5 H,6.6%

Calc. for $C_{20}H_{25}O_3N.CH_3I.H_2O$

C,51.7 H,6.2%

<u>O-Ethylatherospermine</u>. Atherospermine (1.2 g. of crude benzene adduct) was dissolved in methanol (10 c.c.) and ethylated with diazoethane (from 2 g. ethylnitrosourea) in ether. Ethereal diazoethane (from 1 g. ethyl-nitrosourea) was added daily, and after four days the

solvents were removed under reduced pressure and the residue dissolved in hydrochloric acid (100 c.c. ; 1%). The non-phenolic base was precipitated by the addition of sodium hydroxide (1%), extracted with ether and the latter solution dried over sodium sulphate. The ether was removed and the alkaloid again precipitated from the acid by sodium hydroxide to remove any adhering phenolic base. An ether solution of the precipitate was dried (sodium sulphate) reduced to small volume and set aside when O-ethylatherospermine crystallised. Recrystallisation of this base from ether or methanol produced needles (1.0 g.) m.p. 186-188°, undepressed by admixture with O-ethylberbamine, $[\alpha]_D^{18}$= +129 (c, 0.3 in $CHCl_3$ in 4 dcm. tube).

Found

C,73.6 H,6.9 O,15.6 N,4.7%

Calc. for $C_{39}H_{44}O_6N_2$

C,73.6 H,6.9 O,15.1 N,4.4%

Fission of O-Ethylatherospermine. O-Ethylatherospermine (0.8 g.) was dissolved in toluene (10 c.c.) and cleaved in liquid ammonia (300 c.c.) with sodium (0.6 g. in all) the latter being added piece-wise with constant stirring. The ammonia was allowed to evaporate overnight

and the mixture separated into phenolic (0.42 g.) and non-phenolic (0.36g.) fractions by the usual means.

The non-phenolic base was dissolved in benzene and chromatographed on alumina to purify it. Crystallisation proved difficult so its methanolic solution was warmed with excess methyl iodide when colourless needles of O-ethylarmepavine methiodide separated out on cooling m.p. 195-7°, $[\alpha]_D^{18}$= -80.1 (c, 0.2 in CH_3OH in 4 dcm. tube). No depression of melting point was noted when this compound was mixed with the methiodide obtained from the non-phenolic fraction of the O-ethylberbamine fission or with an authentic sample of (-)O-ethylarmepavine methiodide prepared as previously described.

Found

C,54.3 H,6.3 O,10.7%

Calc. for $C_{21}H_{27}O_3N.CH_3I.\frac{1}{2}H_2O$

C,54.2 H,6.3 O,10.7%

The phenolic base was methylated with diazomethane and afforded an oil which was chromatographed and converted to its methiodide m.p. 132°. A mixed melting point with an authentic specimen of (+)O-methylarmepavine methiodide obtained from the phenolic fraction of the fission of O-methylatherospermine showed no depression.

Found

C,51.0 H,6.6%

Calc. for $C_{20}H_{25}O_3N.CH_3I.1\frac{1}{2}H_2O$

C,50.8 H,6.3%

Atherospermine dimethiodide. Atherospermine (1 g.) was refluxed with methyl iodide (1 c.c.) in methanol (10c.c.) for 15 minutes. Removal of the solvents under reduced pressure yielded a resin which could be crystallised from a methanol or ethanol solution, m.p. 230°d.

Atherospermine dimethochloride. Atherospermine (12 g. of thrice crystallised benzene adduct) was dissolved in acetone (50 c.c.) and a solution of methyl chloride in acetone added (20 g. in 20 c.c.). This mixture was placed in a bomb type calorimeter which was heated in a boiling water bath for 3 hours. After cooling overnight in a refrigerator the calorimeter was opened and the solid material (12 g.) removed from the solution by filtration. Atherospermine dimethochloride recrystallised from an acetone-methanol mixture had $[\alpha]_D^{18} = -49$ (c, 0.5 in CH_3OH) and decomposed slowly when heated above 230°.

Found

C,60.8 H,6.9 O,19.8 N,3.3 Cl,9.8 CH_3O,11.8%

Calc. for $C_{37}H_{40}O_6N_2.2CH_3Cl.3\frac{1}{2}H_2O$

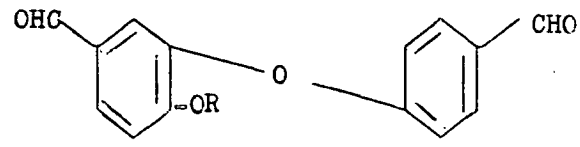
C,60.6 H,6.9 O,19.8 N,3.6 Cl,9.2 $3CH_3O$,12.0%

PART 3

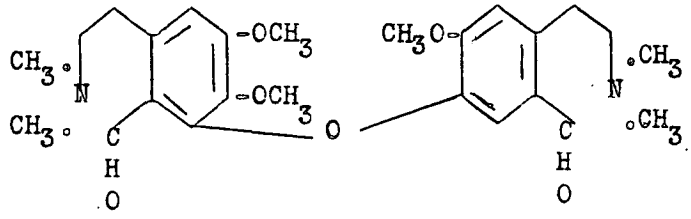
A REINVESTIGATION OF THE BARBERRY ALKALOIDS AND SOME STEREOCHEMICAL PROBLEMS ASSOCIATED WITH THEM

The botanical family Berbidaceae has a very widespread distribution and its members have been long known to provide a rich source of alkaloids of the benzyl and bisbenzylisoquinoline type. As long ago as 1837 Buchner and Herberger (24) isolated the yellow base berberine from the root bark of the common barberry, Berberis vulgaris, while in 1886 Hesse (25) discovered two colourless alkaloids oxyacanthine and berbamine in the species. Since that time further bases have been isolated from the barberry and degradative experiments carried out to elucidate the structure of all compounds.

Following the usual degradative methods, O-methyloxyacanthine dimethiodide when subjected to Hofmann degradation and ozonolysis yielded 2-methoxydiphenyl ether -5:4'-dialdehyde (3.1, R= CH_3) and a diaminodialdehyde which was shown to be (3.11). O-Ethyloxyacanthine dimethiodide on degradation gave compound (3.11) and 2-ethoxydiphenyl ether -5:4'-dialdehyde (3.1, R= C_2H_5) which fixed the position of the hydroxyl group. From this it is evident that oxyacanthine should have either formula



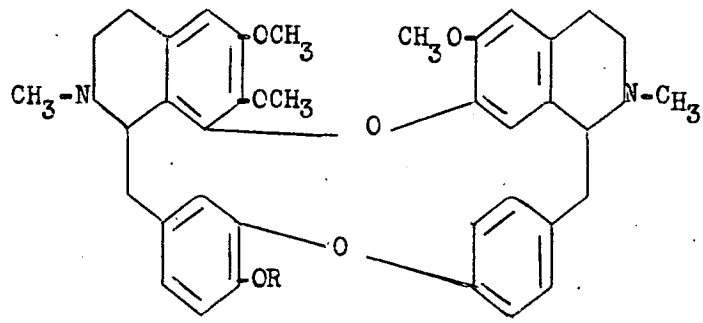
3.1



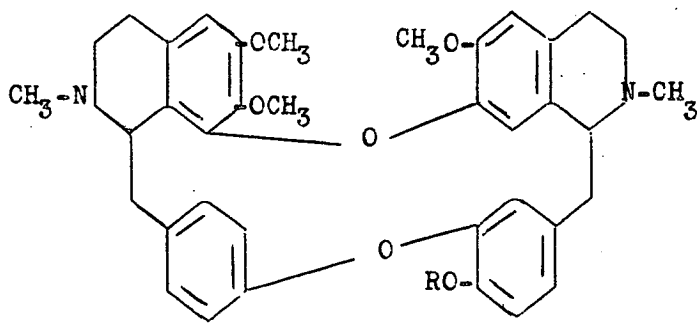
3.11

(3.111 or 3.1V, R= H). The work of the Japanese in solving this ambiguity has been mentioned previously in this thesis; as a result of their degradative fission with sodium in liquid ammonia it is now known that oxyacanthine is (3.1V, R= H).

Von Bruchhausen had shown that O-methylberbamine dimethiodide on degradation gave the same diphenyl ether dialdehyde (3.1, R= CH_3) and diaminodialdehyde (3.11) as oxyacanthine, and since he had proved oxyacanthine to have one of the formulae (3.111 or 3.1V, R= H) the other



3.111



3.1V

was assigned to berbamine. However von Bruchhausen did not repeat the degradation on O-ethylberbamine dimethiodide presumably due to lack of material. Hence the location of the hydroxyl group in berbamine was not proved but was assigned the corresponding position to that of its analogue oxyacanthine. On this assumption the Japanese work indicates the formula (3.111, R= H) for berbamine.

Inubushi (105) has recently applied the sodium cleavage method to berbamine itself. He showed that only one ether link is cleaved initially, but if the amorphous phenolic product is methylated a second cleavage can be achieved to yield (-)O-methylarmepavine (3.V11 , R= CH_3) and (+)armepavine (3.V11, R= H). The production of these compounds indicates that the ether link between the benzyl portions of the molecule is the more resistant to cleavage. It had previously been shown by Tomita, Inubushi and Niwa (104) that ortho and para hydroxydiphenyl ethers are not cleaved by sodium in liquid ammonia, and on the basis of this fact Inubushi has located the hydroxyl group of berbamine ortho to the resistant ether link, i.e. in the benzyl portion of the molecule. In the absence of further model experiments, particularly on hydroxybisdiphenyl ethers, this conclusion is in the author's opinion open to question, since there is an alternative explanation of the Japanese results to the assumption of a specific inhibitive effect of an ortho hydroxyl group. In a simple diphenyl ether, the presence of a phenolic group would lead under the conditions of the sodium-cleavage reaction to the formation of a sodium salt insoluble in the benzene-toluene reagent used, so that the reaction stops due to the insolubility of the material. A single hydroxyl group in a bisbenzyliso-

quinoline alkaloid such as berbamine however is cryptophenolic (thus berbamine is not extractable from chloroform solution with alkali) and the alkaloid is sufficiently soluble for cleavage of one ether link to take place, which would be expected to be the more highly activated one between the isoquinoline residues. This cleavage however produces a second phenolic group, which results in the precipitation of the material as a sodium salt. Methylation of the two phenolic groups then permits further cleavage to take place. If this interpretation of the Japanese experiments, which appears equally valid, is accepted, then they cannot be regarded as furnishing proof of the position of the hydroxyl group, and more definite evidence of its location would seem desirable.

In 1929 von Bruchhausen and Schultze (26) while endeavouring to prepare the monohydrochloride of oxyacanthine by treating the base with an equimolar solution of acid obtained a new base m.p. 260°, $[\alpha]_D^{20} = -94.9$ (CH_3OH). Recently Bick and Todd (21) indicated that this base was in fact identical with repandine, an alkaloid isolated from Daphnandra repandula (27) and shown to be a diastereoisomer of oxyacanthine. This observation prompted von Bruchhausen (106) to suggest that the ethanolic hydrochloric acid used in preparation of the

monochloride had caused a Walden Inversion to occur in one of the centres of asymmetry present in oxyacanthine. Bick and Todd on the other hand using purified oxyacanthine were not able to repeat von Bruchhausen's experiment and they postulated that the repandine was actually present in the original Berberis sp. from which the oxyacanthine was isolated and also as an impurity in von Bruchhausen's sample of oxyacanthine.

The possibility of a Walden Inversion in the oxyacanthine-repandine group of bases was again brought to the fore recently when Tomita et al. (9, 10) reported that O-methyloxyacanthine when cleaved in liquid ammonia with sodium gave a (+)coclaurine derivative together with a (-)coclaurine derivative while O-methylrepandine provided two (+)coclaurine bases. An examination of the magnitude and sign of rotation of the original bases suggested that repandine, $[\alpha]_D$ = -106, should have (+-) centres while oxyacanthine, $[\alpha]_D$ = +279, should have (++) centres. In all other alkaloids cleaved by this method the coclaurine derivatives containing the expected type of centre were obtained and the Japanese explained this apparent anomoly in the cases of oxyacanthine and repandine by assuming that a Walden Inversion occurred during the cleavage of both bases. Since the repandine used by the Japanese was not a natural base but obtained

from oxyacanthine by von Bruchhausen's method it seemed highly desirable, in view of the unexpected result, to repeat the fission experiments on this alkaloid using repandine obtained from natural sources.

From this preamble it can be seen that although the Berberis alkaloids have received constant attention for the last century, numerous problems associated with these bases still remain unsolved. Thus the present author undertook a reinvestigation of the alkaloids present in Berberis vulgaris for the following reasons.

First, a small quantity of berbamine was required for comparison purposes with atherospermine mentioned in the previous section ; a larger quantity was necessary in order to fix unequivocally the position of the phenolic group in berbamine. Secondly, oxyacanthine was required to repeat the fission experiment of the Japanese and finally a search was intended to establish the presence or otherwise of repandine in Berberis vulgaris.

The extraction procedure adopted for the treatment of the ground barberry bark followed the usual pattern ; however there was one important modification. As mentioned above one aim of this investigation was to attempt an isolation of repandine and since von Bruchhausen had claimed that oxyacanthine was converted to

this base by an acid treatment it was decided to follow a procedure without employing acids. After a preliminary extraction with petroleum ether to remove fats the bark was exhaustively extracted in a Soxhlet with methanol. At this stage the methanol solution was divided into two equal portions. One half had the methanol replaced with dilute hydrochloric acid - this was to act as a standard comparison with the other half where the solvent was replaced by dilute aqueous ammonia. The acid extract was allowed to stand for several weeks in a refrigerator to allow any non-basic material to settle out. At the same time the major part of the berberine crystallised as the hydrochloride and was subsequently purified by recrystallisation from ethanol. The aqueous acid solution was made alkaline with ammonia and the precipitated bases taken up in chloroform. The latter solution after a sodium hydroxide extraction to remove phenolic bases was washed and dried and the chloroform replaced with methanol. At this stage the solution was set aside.

The alkaline half was filtered to remove the precipitated bases which after drying were dissolved in chloroform. The chloroform solution was treated as before, the chloroform was replaced with methanol and the

solution set aside.

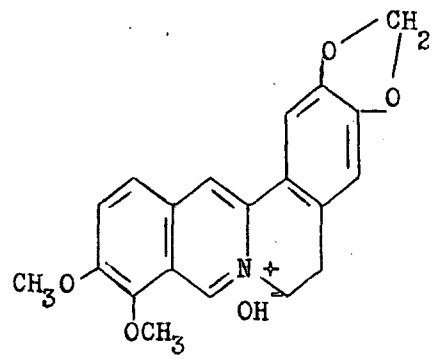
Actually this dual extraction was unnecessary because no repandine was found and the alkaloid content of both halves was identical. The percentage composition tended to be higher in the case of the acid extract but this was due no doubt to the fact that the employment of acid renders purification more efficient and hence increases the yield of alkaloids.

However this rather unusual method of extraction did indicate the presence in the barberry bark of at least one well crystalline non-basic compound. The methanol solution, obtained from the alkaline fraction, when allowed to stand deposited a crystalline precipitate which when dissolved in benzene and chromatographed could be separated into a crystalline yellow non-basic compound and the alkaloid berberine. This non-basic compound which proved to be very unstable even at room temperature was shown to be an amide. Acid hydrolysis gave a reaction mixture which produced a strong Meyer's test (substance prior to hydrolysis gave a negative Meyer's test) indicating the presence of an alkaloidal substance ; separation of this compound was carried out, after making the solution basic with ammonia, by a repeated chloroform extraction. Purification was afforded by partition chromatography on a cellulose column after which the base crystallised from

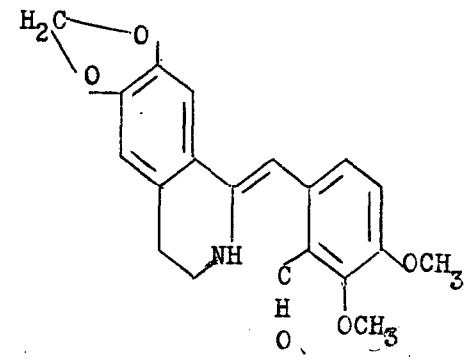
a methanol solution. Its yellow colour, solubility in water, zero rotation, presence of a methylenedioxy group (Labat test) and instability to heat suggested that this compound was berberine. However lack of physical constants together with the small amount of material at hand made a comparison difficult. The identity of the two was eventually indicated by paper chromatography when the unknown base, berberine, and a mixture of the two were all shown to have the same R_f value and the identity was confirmed by a comparison of the ultra-violet spectra of the two compounds.

The alkaline aqueous phase which remained following the chloroform extraction would not yield a crystalline acid. However treatment with calcium chloride gave a crystalline precipitate insoluble in acetic acid but soluble in dilute mineral acids. This indicated the presence of oxalic acid in the aqueous solution and this was substantiated by the fact that the crystalline calcium salt when dissolved in dilute sulphuric acid decolorised a dilute aqueous solution of potassium permanganate. Hence it appeared that the original compound was an amide of berberine and oxalic acid and furthermore since it possessed no acidic properties it must have been diberberine oxalamide.

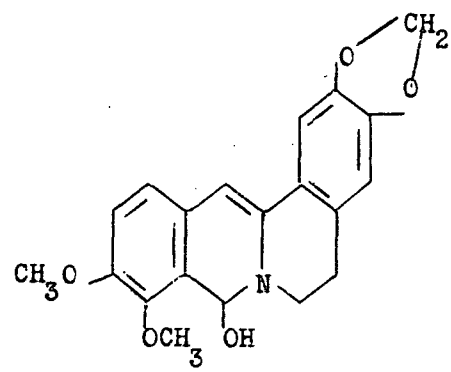
The properties of berberine (32) require the postulation of three theoretical forms for this base (3.X11, 3.X111, 3.X1V). Two of these have actually been



3.X11



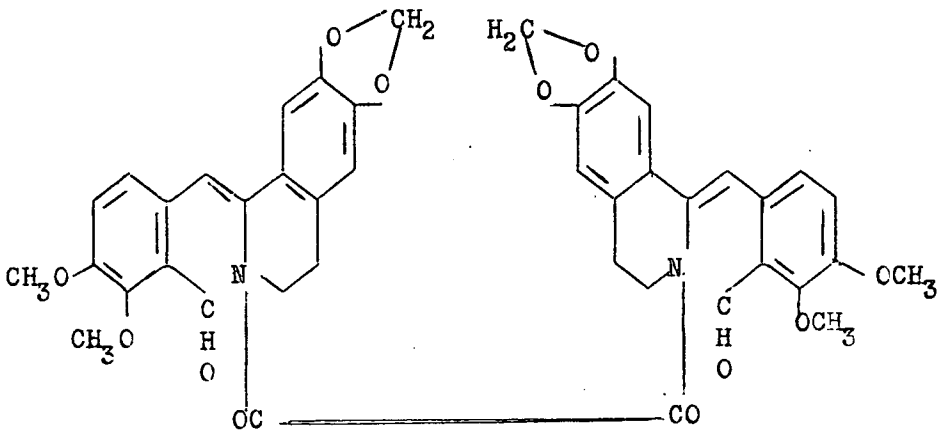
3.X111



3.X1V

obtained ; (3.X11), the ammonium form in which berberine exists in solution when the calculated amount of barium hydroxide is added to an aqueous solution of the sulphate, or when berberineacetone is decomposed by superheated steam and (3.X1V), the carbinol form which represents the original alkaloid first isolated in the pure state and which represents the form of the base when

dissolved in organic liquids. The aldehyde (3.X111) has not been obtained but an oxime has been prepared (72). For berberine to form an amide the aldehyde form (3.X111) must be involved and hence the formula of diberberine oxalamide would be given by (3.XV). Such a



3.XV

formula requires C_{42} H_{36} O_{12} N_2. However the analytical results suggested that the molecule contained a higher percentage of oxygen and hydrogen. This was probably due to solvent of crystallisation which could not be removed owing to the instability of the amide. The analytical figures for a sample crystallised from ethanol agree with the formula C_{42} H_{36} O_{12} $N_2.6\frac{1}{2}H_2O$.

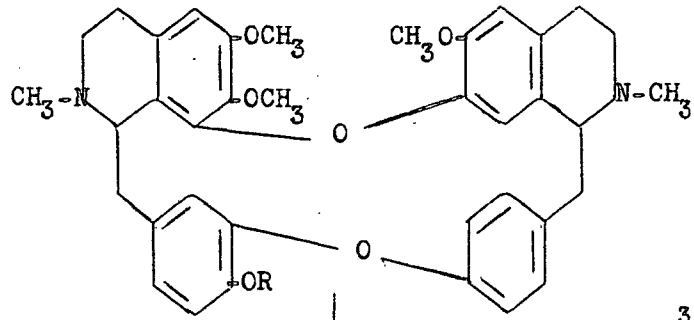
After the removal of the above mentioned crystalline precipitate from the methanol solution the methods used to work up both acid treated and alkali

treated halves were identical. The methanol in both fractions was replaced with benzene and this latter solution was chromatographed on neutral alumina. As a result of this procedure two basic fractions were obtained. The first washed from the column with a solvent consisting of 70% benzene-30% chloroform and was shown to be O-methylberbamine (isotetrandrine). It is of interest to report that this is the first occasion that this base has been found in this species. Such a result strongly indicates that many minor bases may yet turn up in well investigated plants when modern extraction and purification techniques are employed.

The second basic fraction which issued from the column with chloroform as solvent could be separated into two previously recorded bases - oxyacanthine and berbamine. A thorough search however failed to detect the presence of repandine. It should be mentioned however, that when oxyacanthine, isolated from this bark, was submitted to von Bruchhausen's method for conversion to repandine no trace of this latter base could be detected and the oxyacanthine was recovered unchanged.

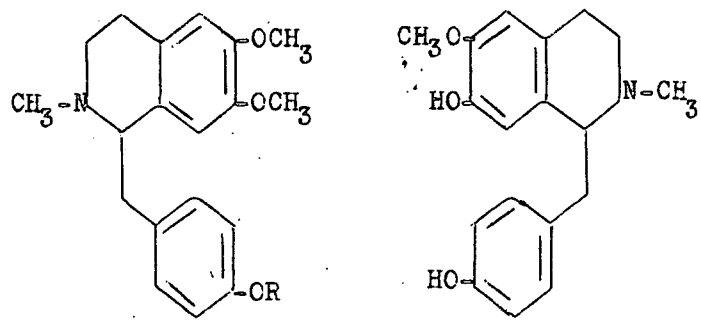
The berbamine after purification by recrystallisation from benzene was ethylated with diazoethane to produce O-ethylberbamine (3.V, R= C_2H_5). This compound, when cleaved with sodium in liquid ammonia gave (-)O-ethyl-

armepavine (3.V11, R= C_2H_5) (identified as its methiodide) and (+)N-methylcoclaurine (3.V1). The melting point of



3.V

sodium
liquid ammonia



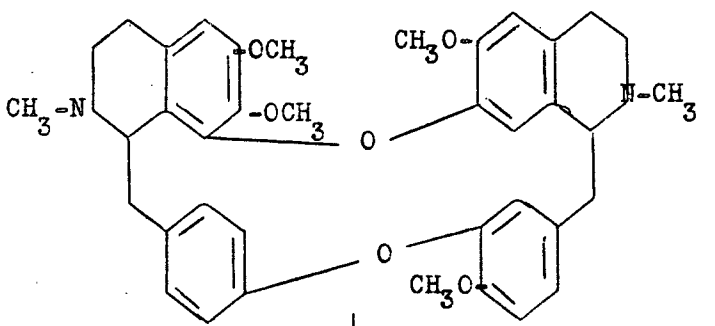
3.V11

3.V1

the former compound was undepressed by the addition of an authentic specimen of O-ethylarmepavine methiodide obtained from the fission of O,O-dimethyl<u>iso</u>chondrodendrine. This indicated that berbamine was (3.V, R= H) and proved unequivocally the location of the hydroxyl group in this base.

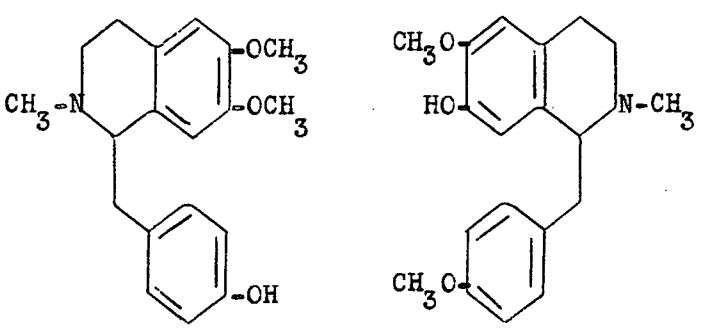
As previously mentioned it was thought desirable to

repeat the reductive cleavage of oxyacanthine and repandine as the results obtained by the Japanese were contrary to expectations. Oxyacanthine (ex Berberis vulgaris) and repandine (ex Daphnandra repandula) were methylated with diazomethane to give the corresponding O-methyl ethers (3.V111). Fission with sodium in liquid ammonia, however, only substantiated the Japanese results ; O-methyloxyacanthine produced (+)armepavine (3.1X) and a laevorotary coclaurine derivative (3.X) while O-methyl-repandine gave (+)armepavine (3.1X) and a dextrorotary coclaurine base (3.X).



3.V111

sodium
liquid ammonia



3.1X

3.X

Thus it would seem that in this oxyacanthine - repandine series of bases, there are two cases where a Walden Inversion appears to occur - firstly there is von Bruchhausen's reported conversion of oxyacanthine to repandine by alcoholic hydrochloric acid and secondly the appearance of fission products of unexpected signs of rotation when these bases are cleaved with sodium in liquid ammonia. The obvious answer in both cases is provided by the inversion theory but a closer examination of the facts reveals that such an explanation is not as satisfactory as may first appear.

The bisbenzyl<u>iso</u>quinoline alkaloids are a group of very closely related bases and the differences which divide them into sub-groups are very slight indeed. Thus it is rather surprising to find an inversion only in the repandine-oxyacanthine class when the other members although structurally closely allied to these bases show no tendency to invert when subjected to the same reagents.

It is interesting to examine more closely von Bruchhausen's method for converting oxyacanthine to repandine because in this procedure the amount of acid employed is critical. Von Bruchhausen used just enough hydrochloric acid to react with one of the basic groups present in the alkaloid. This partial neutralisation

was followed by chloroform extraction and the organic phase so obtained yielded repandine (20% based on original oxyacanthine). However when the amount of acid is increased so both basic groups are neutralised, no repandine has been isolated. It is strange that the amount of acid should have such a critical effect on this reaction if a Walden Inversion were responsible ; rather does it seem probable that both oxyacanthine and repandine were present in the plant extract and the addition of a small amount of acid provides a competitive neutralisation of the two bases. Since repandine appears in the chloroform solution it would seem that this is a weaker base than oxyacanthine which remains in the aqueous phase as the hydrochloride. The fact that repandine actually is a weaker base than oxyacanthine is indicated by their dissociation constants. The value 2.7×10^{-8} for repandine is due to Bick and Whalley (27) while the present author obtained the apparent dissociation constant of oxyacanthine as 5×10^{-8} using a similar method of determination. The following table (28) of R_f values also indicates the difference in basic strength of these compounds. The smaller R_f value of oxyacanthine represents a greater basic strength.

TABLE 3.1

R_f values in BuOH : HOAc : H_2O

	25 : 0.1 : 25	25 : 0.5 : 25	20 : 5 : 25
Repandine	0.39	0.45	0.81
Oxyacanthine	0.33	0.37	0.77

However this competitive effect would be lost when the amount of acid is increased and hence neither base could be extracted into the chloroform phase with the result that no separation is achieved. To substantiate this theory it would be highly desirable to detect the presence of repandine in a Berberis species. The present failure in isolating repandine from Berberis vulgaris coupled with the non-conversion of the oxyacanthine to repandine indicated that this latter base was not present in this species or at least in this variety of species ; alternatively repandine was present in the form of a salt which was very sparingly soluble in methanol.

Von Bruchhausen's and Schultze's original experiment (26) has been repeated by Tomita et al. (10) a few years ago using oxyacanthine from B. thunbergii. However quite recently another Japanese worker reported in a private communication his inability to produce repandine in this way ; Bick and Todd (21) also indicated a

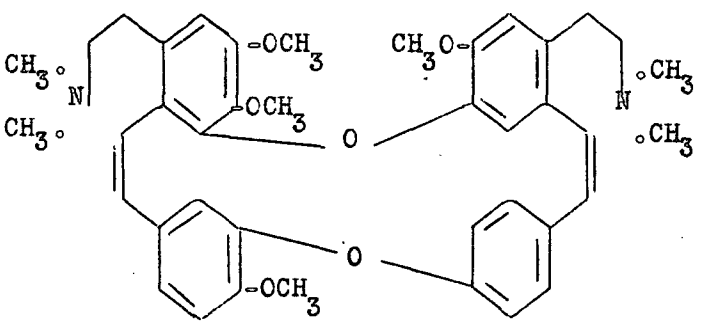
negative result when working with a purified sample of oxyacanthine. These observations coupled with the present author's experience suggests that this conversion is by no means a general rule but that repandine occurs in some Berberis species or sub-species while not in others, or alternatively it has a geographical or seasonal distribution amongst the barberries.

Examination of the reductive cleavages of the O-methyl ethers of oxyacanthine and repandine, where it is claimed another Walden Inversion occurs, again shows frailties in the arguments. In this case it seems rather unlikely that a Walden Inversion would change an asymmetric centre from (+) to (-) in one base and from (-) to (+) in its diastereoisomer both reactions taking place under exactly the same conditions and producing quantitative yields. If such an inversion did occur it would seem more feasible to expect some sort of equilibrium to be obtained consisting of a mixture of (+) and (-) forms of the coclaurine derivative given by formula (3.X). Moreover it seems natural to expect that this equilibrium would be the same whether approached from the repandine or oxyacanthine side. However no such equilibrium is attained ; instead the coclaurine bases produced are optically pure. Thus it is felt that an inversion does

not occur during this reductive cleavage and that oxyacanthine has in fact a (+-) configuration while repandine is of the (++) form. The obvious objection to this explanation is the fact that repandine has a negative rotation ($[\alpha]_D$ = -106) while above is postulated two (+) centres for this base. However when the position is examined more closely and the complexity of the factors affecting the total rotation is realised perhaps this defect in the argument may not appear so important. Also this phenomenon is not without parrallel among this group of bases. Isochondrodendrine which has a rotation of (+50°) can be methylated by diazomethane (29) to 0,0-dimethylisochondrodendrine (cycleanine) having a rotation of (-15°). This latter base has been cleaved by the Japanese workers (8) to produce (-)armepavine in quantitative yields. Hence it would appear that isochondrodendrine has two negative centres while possessing a positive rotation.

Despite the fact there are very slight structural differences within the bisbenzylisoquinoline group of bases (each member having the same basic plan of two coclaurine units) a very wide range of rotations is found varying from over 300° in the bebeerine series (and above this value where secondary nitrogen groups occur) to

values below 20° in the isochondrodendrine series. This variation in rotation is usually attributed to differences in the asymmetry of the whole molecule. However since all bisbenzylisoquinoline alkaloids give an inactive methine base when subjected to a Hofmann degradation this molecular asymmetry cannot be a large contributing factor to the total rotation, for despite the lack of asymmetric centres [a typical such methine is shown in (3,X1)] if molecular asymmetry was a predominant factor



3.X1

these methines would still be optically active. Hence it becomes evident there are other influences (about which nothing is known) governing the optical behaviour of bisbenzylisoquinoline alkaloids. However it is quite apparent that the total rotation of the molecule is not simply the arithmetic sum of the rotations of the two asymmetric centres. Bearing this in mind and the general uncertainty associated with the causes of optical activity

in this group of bases it seems much more reasonable to designate the nature of these centres as (+) or (-) on the basis of cleavage experiments with sodium in liquid ammonia than on arguments derived from the magnitude and sign of rotation of the original base.

More information was sought to substantiate the conclusion that oxyacanthine had (+) and (-) centres of asymmetry while in repandine two (+) centres occurred. The Freudenberg principle has been used very sucessfully in a number of cases to decide the nature of a particular asymmetric centre by comparison with a known standard. This principle is applied by observing the rotation of a number of derivatives of the compound under investigation and comparing these with the rotation of the analogous derivatives made from the standard. This standard must be structurally closely related to the unknown and of course must have an asymmetric centre of known configuration. If the two series of rotations show similar variations it is assumed that the asymmetric centre of the unknown compound has the same configuration as the standard. Another method, which is actually a modification of Freudenberg's principle, whereby the stereochemistry of one compound can be related to that of another structurally similar to it, employs the observation of the rotations of the two substances in a series of solvents of different

polarities. This method has been used in the amino-acid field (88) and has been applied to alkaloids of the benzyl*iso*quinoline group by Leithe (89, 90, 91, 92). More recently this method has been applied by Bick (93) to prove the absolute configuration of morphine. As far as could be ascertained neither of these methods have been previously employed where the compound concerned possessed two asymmetric centres. However it was decided to see if by their use any light could be shed on the problem in hand.

It should be indicated at this stage that the application of Freudenberg's principle to this series of bases is made with a slightly different aim than is usual when only one asymmetric centre is involved. Here there are two such centres and the problem is not so much a determination of the configuration of the particular centre but a decision as to whether the two centres are of the same or opposed configurations.

It has been reasonably well established by sodium in liquid ammonia fission experiments together with the signs and magnitude of rotations of the original compound, that the bases phaeanthine, curine and O,O-dimethyl*iso*-chondrodendrine have (--) centres while atherospermine (berbamine), and chondrocurine have (+-) centres. These

bases then can be used as standards for comparison with repandine and oxyacanthine about which there is doubt concerning the nature of their asymmetric centres. It may appear a little unfortunate for comparison purposes that all the bases have not the same sign of rotation. However this is not as important as it may first appear for it is the magnitude of rotation that is the important factor and the sign may be neglected. This is so since for all bases under investigation it is possible to imagine an enantiomer which has an equal and opposite rotation. It therefore matters little which of the two isomers is considered since it is clear if one of the optical isomers has two like centres then its enantiomer must have two like centres also (the fact that they are of opposite sense is not of interest in the present problem) and this applies equally well if the optically active centres are of opposed configuration.

Table 3.2 shows the molecular rotation of a number of bisbenzyl<u>iso</u>quinoline alkaloids observed in solvents of different polarities. If only the magnitude of the rotations is considered it becomes clear at once that the bases fall into two distinct classes. In the first group which includes oxyacanthine, atherospermine and

TABLE 3.2

ALKALOID	MOLECULAR ROTATIONS OF BASES IN VARIOUS SOLVENTS					
	$(C_2H_5)_2O$	C_6H_6	C_6H_5N	C_2H_5OH	$CHCl_3$	N/10 HCl
Oxyacanthine	+2971	+2470	+2196	+1342	+1714	+1275
Atherospermine	+862	+781	+714	+1495	+689	+366
Chondrocurine	+1830	+1414	+685	+1716	+1029	+1378
Repandine	?(-190)	-364	-616	-570	-683	-817
Phaeanthine	-566	-1279	-1236	-1173	-1863	-1579
Curine	-1490	-1854	-1883	-1675	-1830	-1553
O,O-Dimethyliso-chondrodendrine	?	-60	-373	-123	-93	+55

? Base not soluble enough for an accurate determination

chondrocurine the rotation decreases - increases - decreases as the polarity of the solvent is increased. The second group contains phaeanthine, curine, O,O-dimethyl*iso*-chondrodendrine and repandine and in this case the rotation, in general, increases - decreases - increases as the polarity of the solvent increases. It is interesting that oxyacanthine should be grouped with the alkaloids having (+-) centres while repandine is associated with the bases possessing centres of the same configuration.

If it is desired to compare repandine with the first group of bases it is an advantage to consider (+) repandine instead of the naturally occurring (-)repandine. It is then quite clear that the rotation of (+)repandine varies quite differently from the standard alkaloids in the first group. Thus it would then appear that (+)repandine has not unlike centres as have atherospermine and chondrocurine. If this deduction is correct it then follows that (+)repandine and hence (-)repandine must have two like asymmetric centres - a result which was anticipated from the similarity of repandine's variation in rotation to those of phaeanthine, curine etc. In a similar way it may be shown that oxyacanthine does not belong to the curine - phaeanthine- O,O-dimethyl*iso*-

chondrodendrine group which is added evidence for oxyacanthine having opposed optically active centres.

It must be admitted that some irregularities occur in the above table. The discrepancies that are present can be attributed, at least in part, to slight differences in the structures of the bases concerned - particularly with reference to the number of hydroxyl groups present. Ideally it would have been desirable to perform the observation of optical behaviour on fully methylated bases but this, due to crystallisation difficulties, was impossible. However these discrepancies are not important enough to obscure the general pattern of Table 3.2, in which the bases listed, fall into two distinct classes.

TABLE 3.3

ALKALOID	FREE BASE(B)	B+	O-Me B	O-Me B+	O-Me B.2MeI
Repandine	-106	-158	-73	-107	-132
Oxyacanthine	+278	+201	+289		+56
Atherospermine (Berbamine)	+114	+78	+146		-30

Table 3.3 represents the specific rotations, taken from the literature, of the alkaloids repandine and oxyacanthine together with some of their derivatives.

Unfortunately there are not many cryptophenolic bases sufficiently investigated to serve as comparison compounds. However the figures for atherospermine (berbamine) are included and it can be clearly seen (especially if (+)repandine, which has been shown does not alter the situation at all, is considered) that the variation in specific rotation of this base is analogous with oxyacanthine and not repandine.

It has been mentioned that this is probably the first occasion that Freudenberg's principle has been applied to molecules with two asymmetric centres and perhaps on this ground a little caution should be exercised in accepting results derived from such an extension before it has been tested with a much larger number of compounds than has been possible in this case. Probably the last word on the nature of the optical centres in repandine and oxyacanthine will not be written until the bases have been synthesised, thus removing the last trace of doubt from this question. But from the evidence available at the present time the application of Freudenberg's principle does seem to indicate that oxyacanthine has (+-) centres while repandine has (++) centres a result which is in conformity with the sodium liquid ammonia fission experiments performed on these bases.

EXPERIMENTAL

Extraction of Berberis vulgaris Bark. Bark from Berberis vulgaris, collected at Glenorchy, Tasmania, was dried in an air oven at 40° for 48 hours and milled. Extraction (1.7 Kg. of bark) was performed in a Soxhlet type apparatus using petroleum ether (40-60°) as an initial solvent to remove fats and waxes. After a period of 20 hours the extraction was interrupted and the bark freed from the solvent by exposure to the atmosphere for an extended period. This preliminary extraction was followed by a six day extraction with methanol which removed the alkaloids present in the bark. The methanolic solution (6 litres) was filtered and divided into two equal portions.

First Half - Methanol Replaced with Acid. The methanol was removed under reduced pressure and replaced by aqueous hydrochloric acid (1%). During a week's stand in a refrigerator this aqueous solution deposited a quantity of non-basic material together with berberine hydrochloride. After removal by filtration this salt was purified by repeated recrystallisation from ethanol yielding dull yellow crystals (10 g.) which decomposed on heating above 100°, $[\alpha]_D^{18} = \pm 0$. Conversion to berberine proceeded in the usual way to yield the free

base which also crystallised from ethanol to give yellow needles which slowly decomposed when heated above 100°, $[\alpha]_D^{18} = \pm 0$ (C_2H_5OH).

Found

C,59.0 H,5.8 CH_3O,15.0%

Calc. for $C_{20}H_{19}O_5N.3H_2O$

C,59.0 H,6.2 $2CH_3O$,15.2%

The acid filtrate was made alkaline with ammonia (S.G. 0.88) and the precipitated bases extracted with chloroform. Extraction of this solution with sodium hydroxide (2%) removed phenolic material from the chloroform which was washed with water and dried over sodium sulphate. The chloroform was then removed under reduced pressure and the residue exhaustively extracted with benzene. This latter solution was chromatographed on neutral alumina using the following solvents as eluting media.

Benzene	no basic material removed
90% Benzene ... 10% Chloroform	no basic material removed
80% Benzene ... 20% Chloroform	no basic material removed
70% Benzene ... 30% Chloroform	eluate A
60% Benzene ... 40% Chloroform	no basic material removed
50% Benzene ... 50% Chloroform	no basic material removed
40% Benzene ... 60% Chloroform	no basic material removed

30% Benzene ... 70% Chloroform no basic material removed

20% Benzene ... 80% Chloroform no basic material removed

10% Benzene ... 90% Chloroform no basic material removed

Chloroform eluate B

Methanol eluate C

Eluate A. The basic material washed from the above column with 70% benzene - 30% chloroform crystallised when moistened with a little ethanol. Recrystallisation from acetone produced a base (0.1 g) m.p. 180-1°, $[\alpha]_D^{18}$= +139 (c, 0.2 in $CHCl_3$ in 4 dcm. tube).

Found

C,73.6 H,6.9 O,15.4 N,4.7 CH_3O,19.7%

Calc. for $C_{38}H_{42}O_6N_2$

C,73.3 H,6.8 O,15.4 N,4.5 $4CH_3O$,19.9%

The above physical constants suggested this alkaloid was isotetrandrine m.p. 181-2°, $[\alpha]_D^{19}$= +146 as reported by Kondo and Keimatsu (23). A mixed m.p. determination with an authentic specimen of isotetrandrine confirmed this identity.

Eluate B. This fraction which was removed from the column with chloroform was found to consist of two alkaloids which could not be separated by chromatographic means even when an automatic fraction-cutter was employed.

Separation was achieved however in the following way. The residue remaining after the removal of the chloroform from eluate B was dissolved in warm benzene (30 c.c.) and allowed to stand for several days. During this period berbamine (0.9 g.) crystallised as the benzene adduct, and was subsequently purified by repeated recrystallisation from this solvent when the benzene adduct had m.p. 125-135°d. (depending on the rate of heating) and $[\alpha]_D^{19}$=+113 (c, 0.2 in $CHCl_3$ in 4 dcm. tube). These constants are in agreement with those reported in the literature (30) for berbamine, namely m.p. 127°, $[\alpha]_D$ = +108.6.

Found

C,76.1 H,7.0 O,13.4 N,4.1 CH_3O,12.8%

Calc. for $C_{37}H_{40}O_6N_2.1\frac{1}{2}C_6H_6$

C,76.1 H,6.8 O,13.2 N,4.0 $3CH_3O$,12.8%

The mother liquors from the berbamine crystallisation were diluted with benzene (200 c.c.) and re-chromatographed on alumina. The basic material was eluted with chloroform and a fraction-cutter was once more employed in the hope that any repandine that might be present would be separated from the oxyacanthine. However as previously reported no repandine was isolated; oxyacanthine (2.0 g.) was the only crystalline base

obtained. This was further purified by re-crystallisation from ethanol, methanol and acetone at which stage it had m.p. 208° and $[\alpha]_D^{17.5}$ = +279 (c, 0.4 in $CHCl_3$ in 4 dcm. tube) c.f. Henry (31) who reports m.p. 208-9°, $[\alpha]_D$ = +279 ($CHCl_3$).

Found

C,72.9 H,6.65 O,15.9 CH_3O,15.2%

Calc. for $C_{37}H_{40}O_6N_2$

C,73.0 H,6.6 O,15.8 $3CH_3O$,15.3%

The mother liquors from the oxyacanthine crystallisation were combined and evaporated under reduced pressure to dryness. The residue was taken up in a small volume of acetone, seeded with repandine and allowed to stand. However no further crystallisation ensued.

Eluate C. The eluate yielded a small quantity of berberine (0.1 g.) which was crystallised from methanol. This base had $[\alpha]_D^{18} = \pm 0$ and no definite m.p. as it gradually decomposed above 100°. These observations are in agreement with literature reports (32).

Found

C,58.6 H,5.7 CH_3O, 15.0%

Calc. for $C_{20}H_{19}O_5N.3H_2O$

C,59.0 H,6.2 $2CH_3O$,15.2%

Second Half - Methanol Replaced with Aqueous Ammonia. The methanol was removed under reduced pressure and replaced by aqueous ammonia. The bases and other material precipitated by this procedure were taken up in chloroform (1.5 litres) and the phenolic substances removed by sodium hydroxide (2% in water) extraction. The resultant chloroform solution was washed thoroughly with water and dried over sodium sulphate. The chloroform was removed *in vacuo* and the residue taken up in methanol (200 c.c.) when a crystalline precipitate settled out. This was removed by filtration and dissolved in benzene and this latter solution chromatographed on alumina. The column was washed with the following solvents.

Benzene	eluate D
Chloroform	very little material removed
Methanol	eluate E

Eluate D. The fraction crystallised from benzene to yield a yellow non-basic substance (0.3 g.). Purification could be effected by recrystallisation from benzene, ethanol or methanol. Exposure to the atmosphere for extended periods caused a darkening in colour and heating under melting point conditions resulted in decomposition at 160°. The Labat test (73) for a

methylenedioxy group gave a positive result. A sample crystallised from ethanol analysed as follows.

Found

C,57.5 H,5.8 N,3.0 CH_3O,13.4%

Calc. for $C_{42}H_{36}O_{12}N_2.6\frac{1}{2}H_2O$

C,57.4 H,5.6 N,3.2 $4CH_3O$,14.1%

Hydrolysis of Yellow Non-basic Substance. This yellow compound was boiled with hydrochloric acid (20 c.c. ; 20%) until all the solid material had dissolved. After cooling, the mixture was diluted with water (80 c.c.) and made basic with ammonia. Repeated extraction with chloroform freed the aqueous phase of basic material. The chloroform extracts were combined, dried over sodium sulphate and the solvent removed in vacuo leaving a yellow brown oil. This was dissolved in butanol : water : acetic acid (40:50:10 ; top layer) and subjected to partition chromatography on a cellulose (30 g.) column ; elution with the same solvent removed a bright yellow band from the cellulose. The eluate (100 c.c.) was collected and petroleum ether was added to it (500 c.c. ; 40-60°). An aqueous phase containing the base separated from the organic phase. The aqueous phase was made alkaline with ammonia and the solvent removed

under reduced pressure. The residue when moistened with methanol crystallised in yellow needles, $[\alpha]_D^{18} = \pm 0$ (CH_3OH), which did not melt but slowly decomposed when heated above 100°. The Labat test for a methylenedioxy group was positive. These facts were in accord with the properties of berberine and the identity was further confirmed by paper chromatography of an authentic sample of berberine, the base obtained from the hydrolysis of the amide and a mixture of the two. The chromatography was performed on Whatman No. 1 paper using butanol : acetic acid: water (20:5:25 ; top layer) as solvent for a period of sixteen hours. At the end of that period the paper was dried and the R_f values of the three spots calculated. Each had a value of 0.63. Light absorption of this base in ethanol showed maxima at the following wave lengths :- 2300, 2660, 3370, 3500, 4280 Å. By comparison berberine had maxima at 2300, 2660, 3380, 3500, 4280 Å when its absorption spectrum was observed under the same conditions.

Found

C,59.0 H,5.5%

Calc. for $C_{20}H_{19}O_5N.3H_2O$

C,59.0 H,6.2%

The aqueous alkaline solution, which remained after the removal of basic material with chloroform, was boiled with a calcium chloride solution (5 c.c. ; 10%) when a crystalline precipitate formed. This was removed by filtration and shown to be insoluble in water and acetic acid but readily soluble in mineral acids. A solution of this compound in dilute sulphuric acid decolorised dilute aqueous potassium permanganate. These findings indicate the presence of oxalic acid.

Eluate E. Evaporation of the methanol washings to a small volume resulted in crystallisation of berberine (1.0 g.) from this fraction.

The major part of the bases of this second half of the extraction were present in the methanol filtrate which remained after the removal of the crystalline precipitate referred to above. The methanol was removed in vacuo and the residue taken up in benzene. After filtration this solution was chromatographed on alumina following exactly the same procedure adopted for the acid half of the extraction. As a result of this, further quantities of isotetrandrine (0.06 g.), berbamine (0.6 g.), and oxyacanthine (1.0 g.) were obtained but no indication was found for the occurrence of repandine.

Phenolic Bases. The sodium hydroxide extracts from both portions of the procedure were combined and made acid with hydrochloric acid (36%). The resultant solution was filtered to remove non-alkaloidal material, made alkaline with ammonia (S.G. 0.88) and extracted with chloroform. After drying and removal of the solvent a residue (2.0 g.) consisting of the crude phenolic bases remained ; this was set aside for future investigation.

Reaction of Oxyacanthine with a Limited Amount of Hydrochloric Acid. Oxyacanthine (1 g.) was dissolved in warm ethanol (50 c.c.) and after the addition of aqueous hydrochloric acid (16.75 c.c. ; 0.1N) the mixture was allowed to stand overnight. The alcohol was removed under reduced pressure and was replaced with water (50 c.c.). This latter solution which was not completely homogeneous was extracted repeatedly with chloroform. From the aqueous solution oxyacanthine (0.4 g.) was recovered after basification with ammonia. The chloroform solution yielded a further quantity of oxyacanthine (0.55 g.). No trace of repandine could be detected in either fraction and the oxyacanthine recovered after the experiment was identical in all respects with an authentic sample of the base.

Ethylation of Berbamine. Berbamine (0.8 g. of benzene

adduct) was dissolved in methanol (10 c.c.) and ethylated by ethereal diazoethane (diazoethane from 1 g. of ethylnitrosourea added daily for four days). The solvent was removed under reduced pressure and the residue taken up in dilute hydrochloric acid (100 c.c. ; 1%). The non-phenolic bases were precipitated with sodium hydroxide (1%) with constant mechanical agitation. The flocculent precipitate was taken up in ether and after drying (sodium sulphate) the solvent was removed and the residue once more dissolved in hydrochloric acid. Reprecipitation with sodium hydroxide removed any adhering phenolic base ; the insoluble non-phenol was again dissolved in ether and after drying in contact with sodium sulphate the solvent was reduced to a small volume and the solution set aside when crystallisation followed. Recrystallisation from methanol yielded O-ethylberbamine (0.6 g.) as needles m.p. 185-7°d., $[\alpha]_D^{18}$= +128.3 (c, 0.3 in $CHCl_3$ in 4 dcm. tube).

Found

C,73.4 H,7.1 O,15.7%

Calc. for $C_{39}H_{44}O_6N_2$

C,73.5 H,7.0 O,15.1%

Fission of O-Ethylberbamine. O-Ethylberbamine (0.45 g.) was dissolved in toluene (10c.c.)and cleaved with sodium (0.4 g.) in liquid ammonia (300 c.c.). The fission products were separated in the usual way to yield a phenol (0.18 g.) and a non-phenol (0.23 g.). The latter after purification by chromatography yielded a crystalline methiodide from methanol when boiled with excess methyl iodide, m.p. 195-7°, $[\alpha]_D^{18} = -80.6$ (c, 0.2 in CH_3OH in 4 dcm. tube). This methiodide showed no depression of melting point when mixed with (-)O-ethyl-armepavine methiodide.

Found

C,54.3 H,6.3 O,10.7%

Calc. for $C_{22}H_{30}O_3NI.\frac{1}{4}H_2O$

C,54.2 H,6.3 O,10.7%

The phenolic base was methylated with diazomethane and the resultant non-phenol converted to its methiodide m.p. 134°. A mixed m.p. with (+)O-methylarmepavine methiodide showed no depression.

Found

C,50.9 H,6.4 %

Calc. for $C_{20}H_{25}O_3N.CH_3I.1\frac{1}{2}H_2O$

C,50.8 H,6.3%

O-Methylrepandine. Repandine (1 g.) was dissolved in methanol (300 c.c.) and diazomethane (from 2 g. of nitrosomethylurea) in ether added. At two day intervals three similar additions of diazomethane were made after which the solvents were removed *in vacuo* and the residue dissolved in hydrochloric acid (1%). The solution was made alkaline with aqueous sodium hydroxide (1%) and the precipitated base extracted with chloroform. After drying (sodium sulphate) and removal of the solvent under reduced pressure the residue crystallised when moistened with a little warm methanol. Recrystallisation from methanol yielded O-methylrepandine (0.8 g.) as needles m.p. 210-12°, $[\alpha]_D^{18} = -64$ (c, 0.5 in $CHCl_3$ in 4 dcm. tube). The m.p. was undepressed by admixture with an authentic sample of O-methylrepandine isolated as a minor base from some *Daphnandra* sp. (33). Bick, Taylor and Todd (33) report m.p. 212°, $[\alpha]_D^{15} = -70.5$ ($CHCl_3$) for this base ; Tomita *et al*. (10) record m.p. 210-13°, $[\alpha]_D^{26} = -80.4$ ($CHCl_3$).

Found

C,73.3 H,6.8 CH_3O,19.6%

Calc. for $C_{38}H_{42}O_6N_2$

C,73.3 H,6.8 $4CH_3O$,19.9%

Fission of O-Methylrepandine. O-Methylrepandine (0.7 g.) was dissolved in benzene-toluene (40 c.c. ; 1:1) mixture and the solution added to liquid ammonia (500 c.c.). The cleavage with sodium (1.3 g.) added piece-wise resulted in the production of non-phenolic (0.08 g.) and phenolic (0.6 g.) fractions which were separated in the usual way. The former proved to be unreacted O-methyl-repandine while the latter could be separated into two phenolic bases by the following procedure. The mixture of phenols in ethanol (5 c.c.) was treated with oxalic acid (0.47 g. in 2 c.c. of ethanol) and set aside. A crystalline precipitate (0.28 g.) appeared which following repeated recrystallisation from ethanol had m.p. 210° and $[\alpha]_D^{18}$= +80 (c, 0.3 in H_2O). Tomita (10) reports m.p. 212° for the m.p. of armepavine oxalate. The purified oxalate was dissolved in warm water (50 c.c.) and the free base precipitated with aqueous ammonia. Extraction with ether, drying over sodium sulphate and removal of solvent under reduced pressure yielded a crystalline base m.p. 143°. Tomita (10) reports m.p. 145° for the base armepavine. Methylation of this base with diazomethane produced O-methylarmepavine which as before was identified as the methiodide m.p. 136°, $[\alpha]_D^{18}$= +118 (c, 0.3 in CH_3OH), undepressed by admixture

with (+)O-methylarmepavine methiodide.

Found

C,50.2 H,6.4 O,14.9%

Calc. for $C_{20}H_{25}O_3N.CH_3I.1\frac{3}{4}H_2O$

C,50.4 H,6.3 O,15.2%

The mother liquors which remained following the filtration of the crystalline oxalate were evaporated to dryness and the residue dissolved in warm water. Addition of aqueous ammonia precipitated the basic material which was extracted with ether. After drying (sodium sulphate) and removal of the solvent <u>in vacuo</u> the phenol was gained as an oil (0.26 g.) which failed to crystallise. Methylation with diazomethane in a methanol-ether solvent followed by a chromatographic purification yielded a straw coloured oil which when warmed with methanolic methyl iodide gave O-methylarmepavine methiodide m.p. 132°, $[\alpha]_D^{18}$= +117 (c, 0.3 in CH_3OH) undepressed on admixture with an authentic sample of this compound.

Found

C,50.5 H,6.5 O,14.8%

Calc. for $C_{20}H_{25}O_3N.CH_3I.1\frac{3}{4}H_2O$

C,50.4 H,6.3 O,15.2%

O-Methyloxyacanthine. Oxyacanthine (0.4 g.) was dissolved in methanol (50 c.c.) and methylated with diazomethane following procedures previously described. The O-methyloxyacanthine so formed could not be obtained crystalline but was identified as its methiodide which decomposed without melting when heated above 250°, $[\alpha]_D^{18} = +45$ (c, 0.2 in 50% C_2H_5OH). Gadamer and von Bruchhausen (34) give $[\alpha]_D = +42$.

Fission of O-Methyloxyacanthine. O-Methyloxyacanthine (0.3 g.) was dissolved in toluene-benzene mixture (20 c.c.: 10 c.c.) and the mixture added to liquid ammonia (300 c.c.). Cleavage was performed with sodium (1 g.) and as in the case of the O-methylrepandine fission very little non-phenolic material was separated ; the major part of the reaction product was phenolic (0.28 g.). As previously described the phenols were separated by the addition of oxalic acid which precipitated (+)armepavine oxalate (0.1 g.) m.p. 209° and $[\alpha]_D^{18} = +83$ (c, 0.2 in H_2O). The m.p. of this oxalate was undepressed on admixture with the (+)armepavine oxalate obtained from the repandine fission recorded above.

The oxalate was dissolved in warm water and the free base precipitated with ammonia and extracted with ether. After drying (sodium sulphate), the ether was removed in vacuo and replaced by methanol in which solvent the

armepavine was methylated with ethereal diazomethane during a period of a week. Removal of the excess diazomethane and solvent under reduced pressure left the O-methyl ether of armepavine as an oil which when warmed with methanolic methyl iodide gave the crystalline methiodide (0.05 g.) m.p. 134°, $[\alpha]_D^{18}$= +118 (c, 0.2 in CH_3OH). The melting point of this compound was undepressed on admixture with (+)O-methylarmepavine methiodide but a marked depression occurred when it was mixed with (-)O-methylarmepavine methiodide.

Found

C,50.5 H,6.3 O,15.6%

Calc. for $C_{20}H_{25}O_3N.CH_3I.1\frac{3}{4}H_2O$

C,50.4 H,6.3 O,15.2%

The mother liquors following the filtration of the above armepavine oxalate were evaporated to dryness and the residue (0.13 g.) dissolved in warm water. The free base was liberated with aqueous ammonia and extracted with ether. After drying over sodium sulphate, the solution of the base was combined with ethereal diazomethane. The resultant O-methyl ether was purified by chromatography and identified as its methiodide m.p. 136°, $[\alpha]_D^{18}$= -117 (c, 0.2 in CH_3OH). The melting point of this methiodide was unchanged by

admixture with (-)O-methylarmepavine methiodide but a marked depression occurred with (+)O-methylarmepavine methiodide.

Found

C,50.2 H,6.2 O,14.6%

Calc. for $C_{20}H_{25}O_3N.CH_3I.1\frac{3}{4}H_2O$

C,50.4 H,6.3 O,15.2%

Molecular Rotation Determinations. The molecular rotations listed in Table 3.2 were determined with purified solvents and alkaloids using the D line of sodium and at a temperature of 18°.

PART 4

AN EXAMINATION OF THE TERTIARY ALKALOIDS OF CHONDRODENDRON TOMENTOSUM

Chondrodendron tomentosum is a South American member of the botanical family Menispermaceae and it appears fairly certain (45-50) now that this species (together with closely related ones liable to be confused with it) forms the source of the South American Indian arrow poison known as tube curare. The active principle of gourd curare is probably derived from Strychnos species while the origin of pot curare is still obscure.

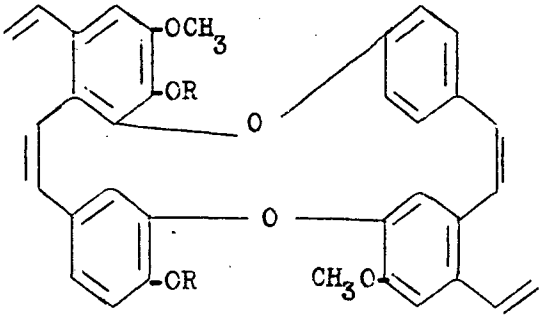
Boehm (51-53) was the first person to undertake a chemical analysis of tube curare and as a result of his work the crystalline base curine and the highly toxic but amorphous tubocurarine were obtained. Later King (46) when examining a sample of tube curare was able to obtain tubocurarine chloride in a crystalline condition and also establish the relationship between it and curine. Quite recently Dutcher (50, 54) has examined a curare prepared by Indians from the Upper Amazon where only plant material from Chondrodendron tomentosum was employed. From this he obtained the following bases - isochondrodendrine, O,O-dimethylisochondrodendrine (cycleanine), curine,

d-tubocurarine chloride and a new base d-chondrocurine. These results seemed to establish definitely that the active principle of tube curare was derived from Ch. tomentosum. However doubt was thrown upon this when King (55) reported the presence of curine and l-tubocurarine chloride in a carefully identified specimen of this plant obtained from Peru. Hence it seems that the botanical description of Chondrodendron tomentosum covers two species containing the d and l forms of tubocurarine chloride or that the basic contents of the plant are not constant.

The present knowledge of the chemistry and structural relationships of the curare alkaloids stems mainly from King. His investigations have also shed much light on the botanical origin of alkaloids in this class. The methods employed by King in degradative experiments on these bases usually involved complete methylation of hydroxyl and amino groups followed by a double Hofmann degradation to produce a nitrogen free compound. The structure of this latter material was determined by permanganate oxidation to yield acids which were compared directly with synthetic samples.

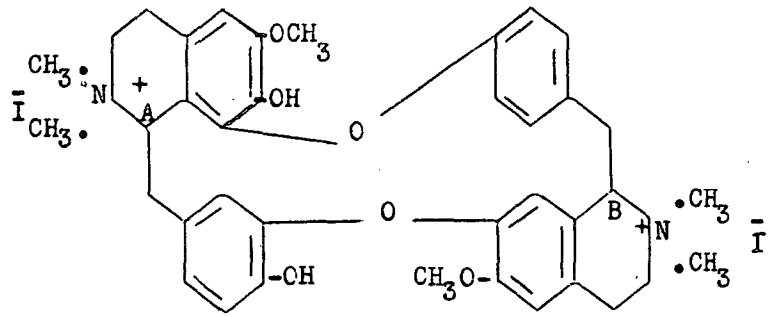
Methylation of bebeerine [(+) curine] (56) with sodium methoxide and methyl iodide in methanol gave an

amorphous O,O-dimethylbebeerine dimethiodide. This when subjected to a double Hofmann gave the nitrogen free O,O-dimethylbebeerilene (4.1, R= CH_3) whose structure



4.1

King determined by oxidation. Ethylation of bebeerine dimethiodide with sodium ethoxide and ethyl iodide produced amorphous O,O-diethylbebeerine dimethiodide. This subjected to a double Hofmann produced O,O-diethylbebeerilene (4.1, R= C_2H_5) (47). Tubocurarine chloride under analogous conditions produced O,O-dimethylbebeerilene (46) and O,O-diethylbebeerilene (49). Hence bebeerine dimethiodide, curine dimethiodide and tubocurarine iodide can all be represented by formula (4.11). The difference between these three bases must be due to the asymmetric centres A and B. Curine and bebeerine are enantiomers and tubocurarine is diastereoisomeric with them. From a consideration of rotations and the nature of methines obtained from



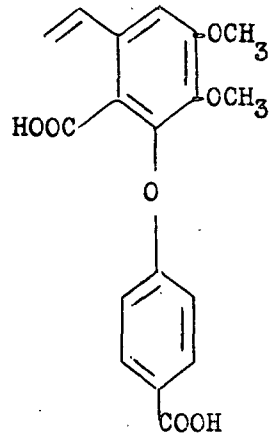
4.11

the first Hofmann degradation King ascribed to bebeerine the (++) configuration, to curine the (--) configuration and to tubocurarine the (+-) configuration.

Dutcher (50) found that chondrocurine dimethiodide was distinct from tubocurarine iodide but that 0,0-dimethyltubocurarine iodide and 0,0-dimethyl-chondrocurine dimethiodide were identical. Hence tubocurarine iodide and chondrocurine dimethiodide must differ only in the positions of the hydroxyl groups in the molecule.

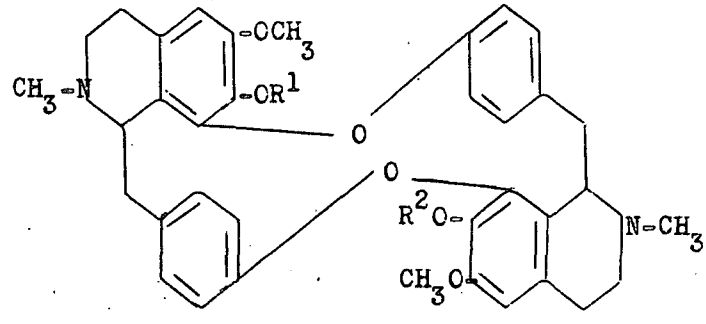
0,0-Dimethyl<u>iso</u>chondrodendrine dimethiodide when subjected to a Hofmann degradation (58, 59) yielded a mixture of methines. The inactive one was separated and subjected to ozonolysis ; hydrogenation of the product followed by oxidation with permanganate and a second Hofmann degradation gave 2, 3-dimethoxy -

6:4'-dicarboxy-5-vinyldiphenyl ether (4.111). The



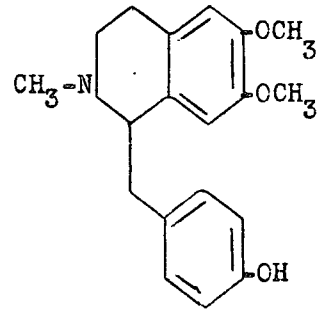
4.111

isolation of this compound in a high yield (64%) indicated that 0,0-dimethyl*iso*chondrodendrine must consist of two identical halves ; hence this base is given by formula (4.1V, $R^1 = R^2 = CH_3$). This result was substantiated by



4.1V

Tomita *et al*. (8) when they cleaved 0,0-dimethyl*iso*-chondrodendrine (cycleanine) with sodium in liquid ammonia and obtained armepavine (4.V) as the only



4.V

reaction product. The position of the hydroxyl groups in isochondrodendrine is however still unsettled. King (48) indicates that one and probably both hydroxyl groups are adjacent to the ether linkages on the basis of his Millon's test (48, 60) which requires a hydroxyl group to be present either in 4' position of the benzyl part of the molecule or the 7 position of the isoquinoline unit. However no chemical evidence was available to substantiate this.

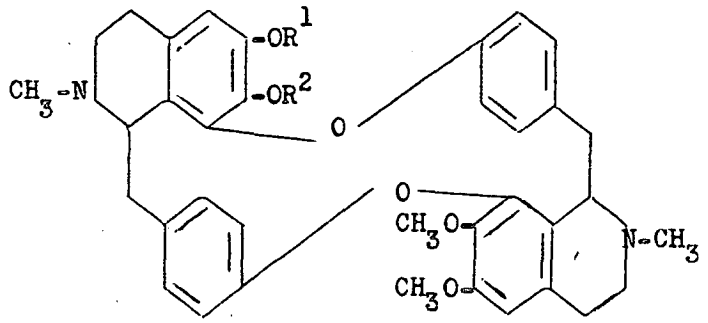
Through the kindness of Dr. Dutcher a large amount of crude tertiary alkaloids from Chondrodendron tomentosum was made available to the present author. It was anticipated that some further information could be gained about the structure of the bases mentioned above (particularly in orientating the hydroxyl groups) from degradative experiments with sodium in liquid ammonia.

Also it was hoped to isolate some of the minor bases that Dr. Dutcher had shown to be present in this species by paper chromatographic experiments.

The crude alkaloid was exhaustively extracted with chloroform and the phenolic bases removed from the solution by shaking with aqueous sodium hydroxide. The phenolic extract was further fractionated into its components by methods described below. The cryptophenols and non-phenols at this stage were present in the chloroform solution. Chromatography of a benzene solution of these on alumina separated this fraction into four compounds. The major component present was shown to be O,O-dimethyl*iso*chondrodendrine previously recorded in this plant by Dutcher (50, 54) and in the Eastern species *Cissampelos insularis* by Kondo and Yano (61, 62). Two further crystalline bases were obtained from this fraction both of which were distinct from O,O-dimethyl*iso*-chondrodendrine and in all probability are new bases. One of these, for which the name tomentosine is proposed, has the formula $C_{37}H_{40}O_6N_2$ making it isomeric with oxyacanthine and berbamine. The analyses showed the presence of three methoxyl groups thus indicating the presence of one cryptophenolic group. The occurrence of such a group was confirmed by the conversion of

tomentosine to its O-methyl ether with diazomethane. The previous isolation of bisbenzyl*iso*quinoline bases from this plant together with the formula of tomentosine strongly indicated that this new base was also a member of this group. Its low rotation, $[\alpha]_D = -45$, further suggested that it may belong to the *iso*chondrodendrine sub-group. This was confirmed by showing that O-methyltomentosine and O,O-dimethyl*iso*-chondrodendrine were in fact identical.

Since the *iso*chondrodendrine molecule consists of two equal halves it follows that the 6 and 7 positions of one *iso*quinoline ring are respectively equivalent to the 6 and 7 positions of the other *iso*quinoline ring system (cf. 4.1V). This reduces by half the number of positions which the phenolic group in tomentosine could occupy and indicates that this base can be represented by (4.V1) where either R^1 or R^2 is H and the other CH_3. However since tomentosine gives a positive Millon's test it seems probable that R^2= H and R^1= CH_3 for then a hydroxyl group would be at the 7 position of the *iso*quinoline ring system (the 4 position of benzyl unit is blocked in both cases). It is interesting to note that this is the first occasion that a cryptophenolic



4.VI

base of this type (chondrofoline has two secondary nitrogen groups as well) has been recorded from the Chondrodendron species.

Recently some workers at the University of Oxford (63) isolated from Chondrodendron limaciifolium a base (designated Base B) whose properties resembled those of tomentosine (see Table 4.1). In addition these workers

TABLE 4.1

	OXFORD BASE B	TOMENTOSINE
m.p.	ca 230°	245°
$[\alpha]_D$ in Chloroform	+31	-45
Ferric chloride	light purple	light purple
Millon's test	positive	positive

quote formula $C_{32}H_{27}O_5N_2(OCH_3)_3$ which is an unprecedented composition for a chondrodendron alkaloid

and difficult to reconcile with a bisbenzyl*iso*quinoline structure. Table 4.2 compares the analyses of these

TABLE 4.2

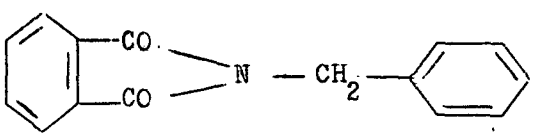
	C	H	CH_3O
Found	68.3	6.2	13.6
Calc. for $C_{32}H_{27}O_5N_2(OCH_3)_3$	68.5	5.9	$3CH_3O$ 15.2
Calc. for $C_{37}H_{40}O_6N_2.2H_2O$	68.9	6.9	$3CH_3O$ 14.4
Calc. for $C_{37}H_{40}O_6N_2.2\frac{1}{2}H_2O$	68.0	6.9	$3CH_3O$ 14.2

English workers with those for a monohydroxyl bisbenzyl*iso*-quinoline base containing water of crystallisation. The present author has found that solvent of crystallisation is very difficult to remove in this series of compounds especially where hydroxyl groups are involved ; complete removal of solvent very often results in partial decomposition of the base. Since the Oxford workers obtained from this *Chondrodendron* species other alkaloids (*iso*chondrodendrine and a base which is probably protocuridine) belonging to the bisbenzyl*iso*quinoline class it seems not unlikely that their Base B is also of that group. Furthermore Table 4.2 suggests it is a monohydroxyl bisbenzyl*iso*quinoline alkaloid and a comparison of the properties of tomentosine and this base

indicates that the two may be enantiomers.

The second new base isolated from Chondrodendron tomentosum was present in only minute amounts. Its melting point 260° resembled tomentocurine, m.p. 265° (57) isolated by King from the leaves of Chondrodendron tomentosum but their identity is doubted since tomentocurine showed marked phenolic properties while the base of m.p. 260° did not appear phenolic.

The fourth compound obtained from the non-phenolic fraction although containing nitrogen proved to be non-basic in character. This compound which was optically inactive and had m.p. 115° analysed for C_{15} H_{11} O_2 N. Hydrolysis experiments indicated that the nitrogen was present in an amide linkage. A literature survey indicated that this compound was in all probability N-benzylphthalimide (4.V11) and this identity was



4.V11

confirmed by a direct comparison of the naturally occurring amide with an authentic sample synthesised after a method described by Ing and Manske (64).

As far as could be ascertained this was the first time that this amide had been found in nature.

The phenolic part which represented the major portion of the alkaloid from Chondrodendron tomentosum proved rather troublesome to separate into its constituents. This was due to the rather large amount of basic material involved and the relative insolubility of some of the phenolic alkaloids present. A benzene extraction of the crude material yielded a partial separation however, for from the resultant solution curine and chondrocurine were obtained in a crystalline form. Dutcher (29) had previously reported the use of ether to separate chondrocurine from the other constituent alkaloids ; this procedure was also attempted but had to be abandoned before the extraction was complete as the emulsions formed were very difficult to handle. However this ether extract did contain basic material which was separated into three crystalline components - curine, chondrocurine and a base whose physical constants resembled those reported by King (57) for tomentocurine isolated by him from the leaves of Chondrodendron tomentosum. The identity of these two bases was confirmed by the fact that an authentic sample of tomentocurine, kindly supplied by Dr. King, did not depress the melting point of the base isolated during

this extraction.

This base tomentocurine analysed as an ordinary diphenolic bisbenzylisoquinoline base, $C_{36}H_{38}O_6N_2$ making it isomeric with chondrocurine and isochondrodendrine. Its specific rotation in acid solution (+202) is very similar to chondrocurine (+200) which suggests that this base may belong to the bebeerine group of bases (4.X) rather than the isochondrodendrine group (4.X1) which has a much lower rotation ($[\alpha]_D$ less than 50). If this is so it is possible that tomentocurine is actually the tertiary base corresponding to tubocurarine chloride. In an effort to prove this an attempt was made to prepare tomentocurine dimethiodide to compare with tubocurarine iodide. However up to this stage tomentocurine dimethiodide has not been obtained in a crystalline condition.

The crude phenolic bases remaining after the benzene and ether treatment consisted of mainly impure isochondrodendrine part of which was purified by recrystallisation from methanol. As mentioned above several features of the structure of these phenolic bases from Chondrodendron tomentosum were left in doubt by degradative methods previously employed. Cleavage

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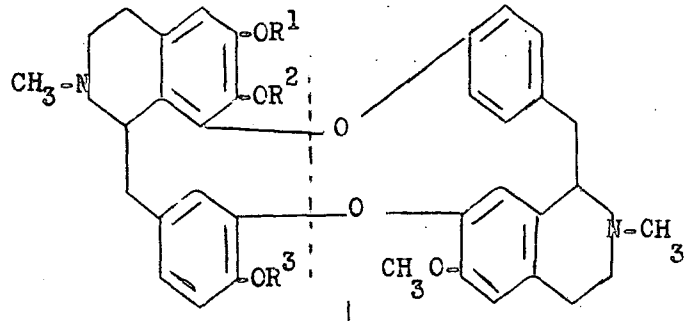
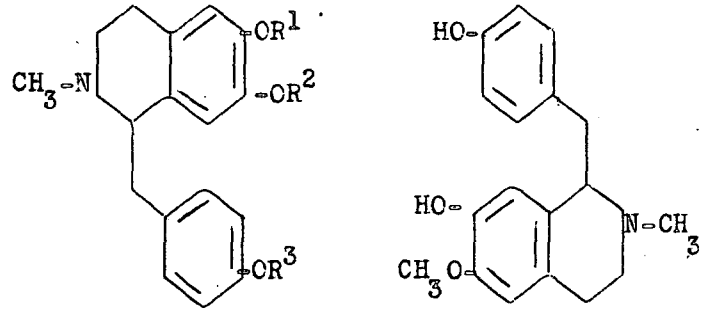
The crude phenolic bases remaining after the benzene and ether treatment consisted of mainly impure *iso*-chondrodendrine part of which was purified by re-crystallisation from methanol. As mentioned above several features of the structure of these phenolic bases from *Chondrodendron tomentosum* were left in doubt by degradative methods previously employed. Cleavage

experiments with sodium in liquid ammonia on their O-methyl and O-ethyl ethers has shed further light on this problem. O,O-Dimethylcurine prepared by the action of ethereal diazomethane on a methanolic solution of curine was cleaved with sodium in liquid ammonia. A non-phenolic and phenolic product resulted which were separated by the usual methods - these were shown to be (-)O-methyl-armepavine (4.V111, $R^1 = R^2 = R^3 = CH_3$) and (-)N-methyl-coclaurine (4.1X). These would be expected as fission products if (4.X, $R^1 = R^2 = R^3 = CH_3$) is the formula for



4.X

sodium
liquid ammonia



4.V111

4.1X

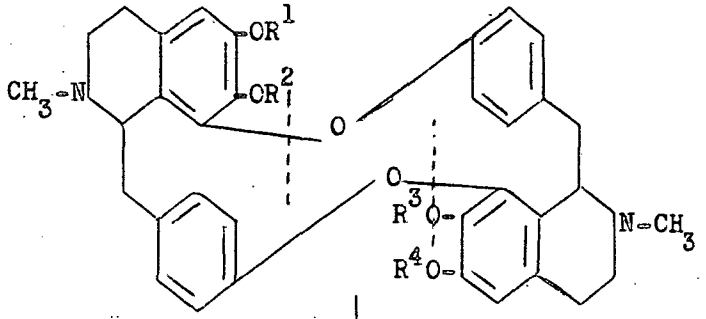
O,O-dimethylcurine which is in agreement with the degradative experiments of King mentioned above. The fact that both fission products are laevorotary indicates the presence of two (-) centres in curine.

Diazomethane methylation of chondrocurine produced O,O-dimethylchondrocurine which when cleaved with sodium in liquid ammonia gave (-)O-methylarmepavine (4.V111, $R^1 = R^2 = R^3 = CH_3$) and (+)N-methylcoclaurine (4.1X). Hence O,O-dimethylchondrocurine is (4.X, $R^1 = R^2 = R^3 = CH_3$) which supports Dutcher's (50) previous finding that O,O-dimethylchondrocurine dimethiodide is identical with O,O-dimethyltubocurarine iodide. The fact that two coclaurine bases of opposing rotation result from this fission indicates the presence of (+) and (-) centres in chondrocurine and also in tubocurarine since the above mentioned identity of the two O,O-dimethyl dimethiodides is firmly established. Ethylation of chondrocurine with diazoethane gave O,O-diethylchondrocurine which when cleaved in the usual way produced (+)N-methylcoclaurine (4.1X) and a non-phenolic base. The latter compound would not crystallise so an attempt was made to prepare its methiodide in order to characterise it. However a crystalline periodide resulted. Boiling with copper

powder gave an oily residue (presumably the methiodide) which also failed to crystallise. Although a periodide may not be a very satisfactory derivative it is clear that this compound carries the two ethoxyl groups present in the original O,O-diethylchondrocurine since the other fission compound has been shown to be the same as the one derived from O,O-dimethylchondrocurine. The presence of the two ethoxyl groups in the periodide was confirmed by analysis. The orientation of these ethoxyl groups has not been established by chemical means but it can be safely assumed that the amorphous non-phenol which resulted from the cleavage of O,O-diethyl-chondrocurine is O,O-diethyl-N-methyl<u>iso</u>coclaurine (4.V111, $R^1 = R^3 = C_2H_5$, $R^2 = CH_3$). The only other alternatives are ($R^1 = R^2 = C_2H_5$, $R^3 = CH_3$) and ($R^2 = R^3 = C_2H_5$, $R^1 = CH_3$). In the former case chondrocurine would be a 1, 2-dihydroxyl compound. However it fails to give a test for a catechol grouping. If the latter orientation were correct then chondrocurine dimethiodide should be identical with tubocurarine iodide ; Dutcher (50) has shown this not to be the case. Hence it follows that chondrocurine can be represented by (4.X, $R^1 = R^3 = H$; $R^2 = CH_3$).

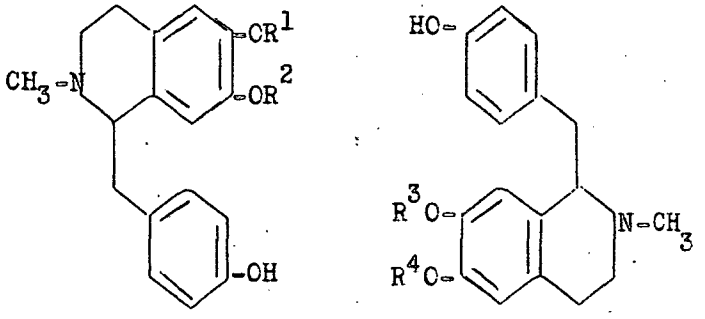
<u>Iso</u>chondrodendrine was ethylated with diazoethane and the resultant diethyl ether cleaved with sodium in

liquid ammonia. From this procedure an amorphous phenolic material was obtained which was methylated with diazomethane. The resultant base could not be obtained crystalline but yielded a crystalline periodide when boiled with methyl iodide in methanol. The interesting point about this series of reactions is that only one periodide was obtained and this in almost quantitative yield. This indicates that only one fission product was obtained in the original cleavage as the methylation of the phenolic group could not alter the number of compounds present since it is known also that O,O-dimethyl<u>iso</u>chondrodendrine yields only one fission product (8). Hence it follows that O,O-diethyl-<u>iso</u>chondrodendrine must be a symmetrical substance and since it is known that O,O-dimethyl<u>iso</u>chondrodendrine (8, 58, 59) is (4.X1, $R^1 = R^2 = R^3 = R^4 = CH_3$) it follows that the diethyl analogue must be (4.X1, $R^1 = R^4 = C_2H_5$ and $R^3 = R^2 = CH_3$ or $R^3 = R^2 = C_2H_5$ and $R^1 = R^4 = CH_3$). Hence the fission product must be (4.X11, $R^1(R^4) = C_2H_5$; $R^2(R^3) = CH_3$ or vice versa). Until the position of the ethoxyl group in this phenol is established the exact formulation of <u>iso</u>chondrodendrine cannot be given. The best that can be said at the moment is that it is (4.X1, $R^1 = R^4 = H$; $R^3 = R^2 = CH_3$ or $R^3 = R^2 = H$; $R^1 = R^4 = CH_3$).



4.X1

sodium
liquid ammonia



4.X11 4.X11

The latter possibility is probably correct for iso-chondrodendrine gives a Millon's test. King's rule for a positive reaction with this reagent requires a free hydroxyl group either in position 4 of the benzyl ring or the 7 position of the isoquinoline ring. Since both 4 positions are blocked it appears likely that both phenolic groups are at the 7 positions.

EXPERIMENTAL

Isolation of Alkaloids. Crude alkaloids (400 g. of crude bases from the tertiary fraction obtained as a by-product during the isolation of tubocurarine chloride) were exhaustively treated with cold chloroform (6 x 500 c.c.). The residue was removed by filtration and repeatedly boiled with fresh quantities of this solvent (4 x 500 c.c.).

Chloroform Insoluble Portion. The solid (35 g.) which remained undissolved after repeated extraction with boiling chloroform still gave strong Meyer's tests and was extracted in a Soxhlet with methanol over a period of several days. During this time crystals appeared in the methanol solution. Repeated recrystallisation from this solvent yielded isochondrodendrine (2.0 g.) as the only crystalline base obtainable from this fraction.

Chloroform Soluble Portion. The chloroform extracts from above were combined (5 litres) and evaporated under reduced pressure to a more convenient volume (1.5 litres). The phenolic alkaloids were removed by extraction with aqueous sodium hydroxide (5% ; 8 x 400 c.c.). The chloroform solution which at this stage contained the cryptophenols and non-phenols was washed thoroughly with water and extracted with hydrochloric acid (1%,

5 x 400 c.c. ; 5%, 2 x 400 c.c.) which removed the majority of the basic materials. At this stage the chloroform solution despite the repeated acid extraction still gave Meyer's tests too strong to be neglected. Hence after drying the chloroform over sodium sulphate the solvent was removed under reduced pressure and the residue taken up in benzene (1 litre). Chromatography on neutral alumina followed and the column was washed with the undermentioned eluting agents.

Benzene	eluate A
80% Benzene ... 20% Chloroform	eluate B
60% Benzene ... 40% Chloroform	no material removed
40% Benzene ... 60% Chloroform	no material removed
20% Benzene ... 80% Chloroform	no material removed
Chloroform	no material removed
Methanol	no material removed.

Eluate A N-Benzylphthalimide. Removal of the benzene left a crystalline deposit (0.2 g.) which when further purified by recrystallisation from petroleum ether (40-60°) had m.p. 115-116° and $[\alpha]_D^{18} = \pm 0$ ($CHCl_3$).

Found

C,76.1 H,4.9 O,13.9 N,6.05% M.Wt,236(Rast)

Calc. for $C_{15}H_{11}O_2N$

C,75.9 H,4.7 O,13.5 N,5.9% M.Wt,237

Preparation of N-Benzylphthalimide. Benzyl chloride (4 g.) was added to an intimate mixture of phthalimide (5 g.) and anhydrous potassium carbonate (1.6 g.) and the whole was heated under reflux for 3 hours. At the end of this period the excess benzyl chloride was removed by steam distillation. Extraction with benzene removed the amide and following drying over sodium sulphate this solution was purified by chromatography on an alumina column. N-Benzylphthalimide (2.8 g.) was obtained from glacial acetic acid in a crystalline condition. A small amount crystallised from petroleum ether (40-60°) had m.p. 115° which was undepressed on admixture with the substance isolated as described above from Chondrodendron tomentosum. Ing and Manske (64) report m.p. 116° for the m.p. of N-benzylphthalimide.

Eluate B O,O-Dimethylisochondrodendrine (Cycleanine). Removal of the solvent left a crystalline reside (1.5 g.) which when further purified by recrystallisation from acetone had m.p. 271-2° and $[\alpha]_D^{18} = -15$ (c, 0.4 in $CHCl_3$ in 4 dcm. tube) and $[\alpha]_D^{18} = -31.9$ (c, 0.4 in C_2H_5OH in 4 dcm. tube). Dutcher (50) reports 269-270° and $[\alpha]_D = -15$ ($CHCl_3$) and Henry (70) gives $[\alpha]_D = -36.8$ (C_2H_5OH) as the physical constants for this base.

Found

C,73.6 H,6.85 O,15.6 N,4.2 CH_3O,19.7%

Calc. for $C_{38}H_{42}O_6N_2$

C,73.3 H,6.8 O,15.4 N,4.5 $3CH_3O$,19.9%

Cryptophenols and Non-phenols. The majority of the cryptophenolic and non-phenolic bases were extracted from the chloroform solution with dilute hydrochloric acid. During a period of several days this acid solution deposited non-alkaloidal material which was removed by filtration. The aqueous filtrate was made alkaline with ammonia (S.G. 0.88) and the precipitated bases filtered off. In order to purify this basic fraction further, it was taken up again in dilute hydrochloric acid (1%) and reprecipitated and refiltered. After drying in vacuo at 50° the crude alkaloid (20 g.) was dissolved in benzene (1 litre) and the solution, filtered from a little suspended matter, was chromatographed on neutral alumina. Elution was effected with the following solvents.

Benzene	no material removed
80% Benzene ... 20% Chloroform	eluate C
60% Benzene ... 40% Chloroform	no material removed

40% Benzene ... 60% Chloroform	eluate D
20% Benzene ... 80% Chloroform	no material removed
Chloroform	eluate E
Methanol	no material removed

Eluate C 0,0-Dimethylisochondrodendrine. Removal of the solvent left a crystalline base (15 g.) which was further purified by recrystallisation from acetone. This had m.p. 270-2°, $[\alpha]_D^{18}$= -15 (c, 0.5 in $CHCl_3$ in 4 dcm. tube) and was undepressed by admixture with a sample of 0,0-dimethylisochondrodendrine obtained from eluate A as described above.

Eluate D. 40% Benzene - 60% chloroform removed from the column a very small amount of basic material (0.01 g.) which crystallised when moistened with acetone. This base had m.p. 255-260° but shortage of material did not allow any further data to be obtained.

Eluate E Tomentosine. Chloroform removed from the column an alkaloid (0.5 g.) which was further purified by recrystallisation from methanol. Tomentosine had m.p. 245°d. (slight darkening above 200°), $[\alpha]_D^{18}$= -45 (c, 0.3 in $CHCl_3$ in 4 dcm. tube). The Millon's test performed on this base gave a positive reaction while the dry base produced a purplish colour when moistened with

ferric chloride.

Found

C,70.3 H,6.65 O,17.7 NCH_3,8.7 CH_3O,14.5%

Calc. for $C_{37}H_{40}O_6N_2.H_2O$

C,70.9 H,6.8 O,17.9 $2NCH_3$,9.3 $3CH_3O$,14.9%

O-Methyltomentosine. Tomentosine (0.3 g.) was dissolved in methanol (200 c.c.) and methylated with ethereal diazomethane (1 g. of nitrosomethylurea). Three further such quantities of diazomethane in ether were added at equal intervals over a period of a fortnight. Removal of the solvents in vacuo left an oily residue which was dissolved in benzene and purified by chromatography on neutral alumina. Elution with benzene-chloroform (3:1) gave O-methyltomentosine (0.2 g.) which was crystallised from acetone. This latter base had m.p. 273°, $[\alpha]_D^{18}$= -16 (c, 0.2 in $CHCl_3$ in 4 dcm. tube) and its m.p. was undepressed on admixture with O,O-dimethylisochondrodendrine.

Found

C,72.1 H,7.0 CH_3O,18.4%

Calc. for $C_{38}H_{42}O_6N_2.\frac{1}{2}H_2O$

C,72.2 H,6.9 $4CH_3O$,19.7%

Phenolic Bases. The aqueous sodium hydroxide solution which removed the phenolic bases from the original chloroform solution was made acid with hydrochloric acid and set aside for several days. During this period a quantity of non-basic material settled out and this was removed by filtration. The acid filtrate was basified with ammonia (S.G. 0.88) and the precipitated alkaloids filtered from the solution. To effect a further purification the bases were re-extracted with hydrochloric acid (1%) the solution again filtered and the alkaloids reprecipitated. At this stage after drying *in vacuo* at 50°, the phenolic bases weighed 230 g.

Benzene Extraction of the Bases. The dried phenolic bases were extracted several times with boiling benzene (8 x 500 c.c.) and the extracts were combined, reduced in volume *in vacuo* and the solution set aside. The benzene adduct of curine (30 g.) crystallised from this solution. The benzene mother liquors remaining after the above adduct had been removed by filtration still contained a large amount of alkaloid. The solvent was removed *in vacuo* and the residue taken up in dilute hydrochloric acid (1%). After filtration the solution was made alkaline with ammonia and the alkaloids extracted with chloroform. This latter solution was

dried over sodium sulphate and the solvent removed under reduced pressure. Fractional crystallisation of the residue from methanol gave curine (10 g.) and chondrocurine (4 g.).

Ether Extraction of the Bases. The material remaining after the benzene extraction was exhaustively treated with boiling methanol (3 x 1000 c.c.) which dissolved the alkaloidal material. After filtration to remove some insoluble matter [which proved to be calcium oxalate (30 g.)], the methanol was removed *in vacuo* and the alkaloids once more taken up in dilute hydrochloric acid (3 litres ; 1%). Basification with ammonia followed and the precipitated alkaloids were extracted with ether. This procedure proved most laborious as very difficult emulsions formed and so the extraction was interrupted before it had gone to completion. The ether extracts were combined (10 litres), dried over sodium sulphate, and the solvent removed. Fractional crystallisation of the residue from methanol gave curine (10 g.), chondrocurine (7 g.) and a base (0.2 g.) which was in all probability identical with the base tomentocurine isolated by King.

The suspended material, left after the ether extraction, was filtered and a small portion of it (5 g. ; ca 1/20) was purified by repeated recrystallisation from methanol to yield isochondrodendrine (3 g.).

Curine. Curine when crystallised from benzene gave the benzene adduct m.p. 161° ; recrystallisation from methanol resulted in the base having m.p. 213° and $[\alpha]_D^{18}$= -318 (c, 0.8 in C_2H_5OH in 4 dcm. tube). Dutcher (50) reports m.p. 165-7° for the benzene adduct of curine. Henry (70) gives m.p. 214° for curine (when crystallised from methanol) and $[\alpha]_D$ = -298 (C_2H_5OH).

Found

C,71.6 H,6.5 O,17.2 N,4.6 CH_3O,10.3%

Calc. for $C_{36}H_{38}O_6N_2 \cdot \frac{1}{2}H_2O$

C,71.6 H,6.5 O,17.2 N,4.6 $2CH_3O$,10.3%

Isochondrodendrine. Repeated purification by recrystallisation from methanol yielded isochondrodendrine m.p. 315°d., $[\alpha]_D^{18}$= +50 (c, 0.5 in C_6H_5N in 4 dcm. tube). The hydrochloride had m.p. 330° and $[\alpha]_D^{18}$= +120 for the ion (c, 0.5 in H_2O in 4 dcm. tube). Dutcher (50) reports m.p. 305°, $[\alpha]_D^{22}$= +50 (C_6H_5N) and m.p. 282-4°d., $[\alpha]_D^{22}$= +121 (H_2O) for the physical constants of the base

and its hydrochloride (rotation calculated for the ion) respectively. Henry (70) reports 333° as the temperature at which the hydrochloride melts.

Found

C,72.3 H,6.5 N,4.6 CH_3O,10.4%

Calc. for $C_{36}H_{38}O_6N_2$

C,72.7 H,6.4 N,4.7 $2CH_3O$,10.4%

Chondrocurine. Repeated recrystallisation from methanol gave chondrocurine m.p. 232° and $[\alpha]_D^{18}$= +173 (c,0.5 in $CHCl_3$ in 4 dcm. tube) $[\alpha]_D^{18}$= +220(c, 0.5 in 0.1N HCl in 4 dcm. tube). Dutcher (50) gives m.p. 232-4° and $[\alpha]_D^{24}$= +200 (0.1N HCl) as the physical constants for this base.

Found

C,72.3 H,6.5 O,16.5 N,4.5 CH_3O,10.3%

Calc. for $C_{36}H_{38}O_6N_2$

C,72.7 H,6.4 O,16.1 N,4.7 $2CH_3O$,10.4%

Tomentocurine. Recrystallisation from methanol-chloroform gave tomentocurine m.p. 260° and $[\alpha]_D^{18}$= +202 (c, 0.2 in 0.1N HCl in 4 dcm. tube). King (57) gives m.p. 265° and

$[\alpha]_D^4$ = +210 (0.1N HCl) for tomentocurine. The m.p. of tomentocurine was not depressed by an authentic specimen of this base. The positive Millon's test recorded by King was also confirmed.

Found

C,67.1 H,6.6 O,21.5 CH_3O,10.1%

Calc. for $C_{36}H_{38}O_6N_2.2\frac{3}{4}H_2O$

C,67.1 H,6.8 O,21.7 $2CH_3O$,9.6%

O,O-Dimethylcurine. Curine (1 g.) was dissolved in methanol (300 c.c.), diazomethane(from 2 g. of nitrosomethylurea) in ether was added and the mixture set aside for two days after which time a further similar quantity of diazomethane was added. After four such additions had been made the solvents were removed in vacuo and the residue dissolved in hydrochloric acid (1%). The solution was made alkaline with sodium hydroxide (1%) and the precipitated base was extracted with a chloroform-ether mixture (1:4). Chromatography on alumina of a benzene solution of the residue afforded a light coloured oil (0.9 g.) which could not be crystallised. Attempts to crystallise the dimethiodide were equally unsucessful as with other authors.

Fission of O,O-Dimethylcurine. O,O-Dimethylcurine (0.5 g.) was dissolved in a benzene-toluene mixture (20 c.c. : 40 c.c.) and the solution added to liquid ammonia (400 c.c.). The cleavage with sodium (1.5 g. in all, added piece-wise) and the isolation and purification of the fission products were carried out in the same manner as described above for other such reactions. The non-phenolic product (0.2 g.) was treated with methanolic methyl iodide and the crystalline methiodide so obtained had m.p. 132° and $[\alpha]_D^{18} = -115$ (c, 0.3 in CH_3OH).

Found

C,50.8 H,6.4 O,15.0%

Calc. for $C_{20}H_{25}O_3N.CH_3I.1\frac{1}{2}H_2O$

C,50.8 H,6.3 O,14.5%

A mixed m.p. determination with an authentic sample of (-)O-methylarmepavine methiodide showed no depression but a mixture with the corresponding dextro compound melted ca 10° lower.

The phenolic fission product, isolated as before, was methylated with diazomethane and afforded a light coloured oil (0.13 g.) from which the methiodide

m.p. 132° and $[\alpha]_D^{18}$= -116 (c, 0.3 in CH_3OH) was formed as before. A mixed m.p. with an authentic specimen of (-)O-methylarmepavine methiodide showed no depression but the (+) compound gave a marked lowering of m.p. when mixed with this methiodide.

Found

C,51.4 H,6.5%

Calc. for $C_{20}H_{25}O_3N.CH_3I.1\frac{1}{2}H_2O$

C,51.3 H,6.3 %

<u>O,O-Dimethylchondrocurine</u>. Chondrocurine (1 g.) was dissolved in methanol (100 c.c.) and methylated with diazomethane as described for curine. The O,O-dimethylchondrocurine (1 g.) so formed could not be obtained crystalline but on treatment of a sample with methanolic methyl iodide the crystalline dimethiodide separated which when crystallised from methanol had m.p. 230-40°d. and $[\alpha]_D^{18}$= +153 (c, 0.3 in H_2O). Dutcher reported m.p. 266° and $[\alpha]_D^{24}$= +160 (H_2O) for O,O-dimethylchondrocurine dimethiodide.

Found

C,48.2 H,5.8%

Calc. for $C_{40}H_{48}O_6N_2I_2.5H_2O$

C,48.2 H,5.9%

Fission of O,O-Dimethylchondrocurine. O,O-Dimethylchondrocurine (0.85 g.) was dissolved in benzene-toluene mixture (1:1) and cleaved with sodium (1.7 g. in all) in liquid ammonia (500 c.c.) as previously described. The non-phenolic and phenolic fractions were separated and purified as indicated above. The non-phenolic base was warmed with methanolic methyl iodide to convert it into its methiodide which when recrystallised from methanol gave needles (0.32 g.) with m.p. 135° and $[\alpha]_D^{18}$= -118 (c, 0.3 in CH_3OH).

Found

C,50.4 H,6.2 O,15.3%

Calc. for $C_{20}H_{25}O_3N.CH_3I.1\frac{3}{4}H_2O$

C,50.4 H,6.3 O,15.2 %

No m.p. depression was noted when the methiodide was mixed with an authentic specimen of (-)O-methylarmepavine methiodide but a marked depression was observed when it was mixed with the dextro compound.

The phenolic base when treated with diazomethane gave the O,O-dimethyl ether which was purified by chromatography and identified as usual as its methiodide m.p. 134°, $[\alpha]_D^{18}$= +120 (c, 0.3 in CH_3OH).

Found

C,50.1 H,6.4 O,14.9%

Calc. for $C_{20}H_{25}O_3N.CH_3I.1\frac{3}{4}H_2O$

C,50.4 H,6.3 O,15.2%

A marked depression of m.p. resulted from admixture with a specimen of (-)O-methylarmepavine methiodide but no m.p. depression was observed with the dextro compound.

O,O-Diethylchondrocurine. Chondrocurine (1 g.) was dissolved in methanol (100 c.c.) and ethylated with diazoethane (from nitrosoethylurea) following the procedures of previous alkylating experiments.

Fission of O,O-Diethylchondrocurine. O,O-Diethylchondrocurine (0.80 g.) was dissolved in benzene-toluene (10 c.c. : 20 c.c.) and cleaved with sodium (1 g. in all) in liquid ammonia (500 c.c.) as previously described. After the separation of the non-phenolic and phenolic bases in the usual way the former compound (0.37 g.) was purified by chromatography. Warming with methanolic methyl iodide produced a periodide m.p. 210-11°.

Found

C,43.7 H,5.7%

Calc. for $C_{22}H_{29}O_3N.CH_3I_2.\frac{1}{2}H_2O$

C,43.6 H,5.3%

Boiling this periodide in methanol with copper powder produced an oil (presumably the methiodide) which could not be obtained crystalline.

The phenolic base when treated with diazomethane gave the methyl ether which when warmed with methanolic methyl iodide gave (+)O-methylarmepavine methiodide m.p. 133° which was undepressed by admixture with an authentic sample of this compound.

Found

C,50.6 H,6.3 O,14.6%

Calc. for $C_{20}H_{25}O_3N.CH_3I.1\frac{1}{2}H_2O$

C,50.8 H,6.3 O,14.5%

O,O-Diethylisochondrodendrine. Isochondrodendrine (1.0 g.) in methanol (250 c.c.) was ethylated with ethereal diazoethane (from nitrosoethylurea) by methods described previously.

Fission of O,O-Diethylisochondrodendrine. O,O-Diethylisochondrodendrine (0.6 g.) was dissolved in benzene-toluene (10 c.c.: 20 c.c.) mixture and cleaved with sodium (1.6 g.) in liquid ammonia (400 c.c.). A phenolic material (0.6 g.) was separated from the reaction mixture and methylated with diazomethane. When the methyl ether so obtained was warmed with methyl

iodide a crystalline periodide (0.7 g.) resulted which could be purified by recrystallisation from methanol, m.p. 172-3°.

Found

C,40.6 H,5.1%

Calc. for $C_{21}H_{27}O_3N.CH_3I_2.2H_2O$

C,40.9 H,5.3%

PART 5

AN INVESTIGATION OF THE OCCURRENCE AND SOME CHEMICAL ASPECTS OF THE DAPHNANDRA ALKALOIDS.

Trees belonging to the genus Daphnandra (family Monimiaceae) are native to Australia and are found in coastal regions of Queensland and northern New South Wales. The presence of alkaloids in the bark of the Daphnandra species was first reported by Bancroft (83, 84, 85) as long ago as 1887. Later Pyman (79) in 1914 isolated three alkaloids from Daphnandra micrantha ; these he named daphnandrine, daphnoline and micranthine. More recently, Bick and Whalley (27,86,87) investigated three further Daphnandra species from which they isolated repandine and repanduline (D. repandula), daphnoline and aromoline (D.aromatica) and repanduline (D. dielsii). The final Daphnandra species, D. tenuipes, which yielded tenuipine, repanduline, aromoline, de-N-methyltenuipine (leaves only), was investigated by Bick, Taylor and Todd (33) who elucidated the main structural features of most of the bases mentioned above. However it has been mentioned previously that the location of hydroxyl and methylimino groups by the Hofmann degradation-

oxidation method of cleavage has always been a very difficult matter and hence the position of these groups in daphnandrine, micranthine, daphnoline and aromoline has been uncertain. Also the yellow base repanduline was not amenable to Hofmann or Emde degradations, a fact which has held up the elucidation of its structure for want of another method of cleavage. In addition two minor bases have been isolated from the Daphnandra species - tenuipine (D. tenuipes, D. dielsii) and repandinine (D. repandula, D. dielsii) ; the amounts of these isolated however have not permitted the standard degradation experiments to be attempted. Thus it was thought if further quantities of Daphnandra alkaloids were available some of the above mentioned problems might be solved especially if sodium in liquid ammonia reduction cleavages were performed on these alkaloids. Hence extractions were carried out on the bark of D. micrantha, D. aromatica, D. tenuipes, D. dielsii to collect further amounts of these alkaloids.

One unexpected result of extractions performed on Daphnandra micrantha subsequent to Pyman's original investigation has been the inability of later workers to duplicate the results of his isolation work. Pyman found large amounts of daphnoline (1.5%) and

daphnandrine (1.5%) and relatively small amounts of micranthine (0.5%) while more recent extractions have yielded micranthine as the dominant (and usually the only) base. In an effort to repeat Pyman's results, Bick, Taylor and Todd (33) examined bark from D. micrantha in different stages of development, from different localities and in different seasons. Although daphnoline and daphnandrine were found in occasional samples these were present in only small amounts and in all cases micranthine was the dominant basic constituent. Pyman did not indicate where his material was collected and hence it must be assumed that the bark which Pyman investigated must have been from a hybrid species or some different strain of D. micrantha which has not been looked at since ; alternatively there might well be some seasonal, or developmental, or geographical distribution within this species which is not fully evident at present. Pyman's material was collected by the Director of the Sydney Botanic Gardens, and thus presumably came from New South Wales. It is perhaps significant in this connection that the samples found by subsequent workers to contain daphnandrine were of N.S.W. origin, although the amounts found in all cases were much less than Pyman found (see Table 5.1).

TABLE 5.1

NOTE 1. X indicates the bases were isolated during the present investigation.

2. Bases printed in capital letters are the major constituents ; small letters indicate the minor alkaloids.

DAPHNANDRA sp.	LOCALITY	ALKALOID	REFERENCE
micrantha	unknown	DAPHNOLINE DAPHNANDRINE micranthine	79
	Mt. Glorious (Q'land)	MICRANTHINE	33
	Upper Brookfield (Q'land)	MICRANTHINE	33
	Draper's Crossing (Q'land)	MICRANTHINE	33
	Upper Brookfield (Q'land)	MICRANTHINE daphnoline	33
	Wauchope (N.S.W.)	MICRANTHINE daphnoline daphnandrine	33
	Toonumbar State Forest (N.S.W.)	MICRANTHINE daphnandrine	33

DAPHNANDRA sp.	LOCALITY	ALKALOID	REFERENCE
micrantha cont'd	East Dorrigo (N.S.W.)	MICRANTHINE	X
repandula	unknown	REPANDINE REPANDULINE O-methylrepandine repandinine	33
tenuipes	Tweed River (N.S.W.)	REPANDULINE tenuipine aromoline	33
	Whian Whian State Forest No. 173 Lismore (N.S.W.)	REPANDULINE tenuipine (phenolic bases not investigated)	X
dielsii	unknown	REPANDULINE tenuipine repandinine O-methylrepandine	33

DAPHNANDRA sp.	LOCALITY	ALKALOID	REFERENCE
dielsii cont'd	Atherton district (N. Q'land)	REPANDULINE REPANDINE tenuipine O-methylrepandine repandinine (?)	X
aromatica	unknown	DAPHNOLINE aromoline	87
	Atherton district (N.Q'land)	DAPHNOLINE	X

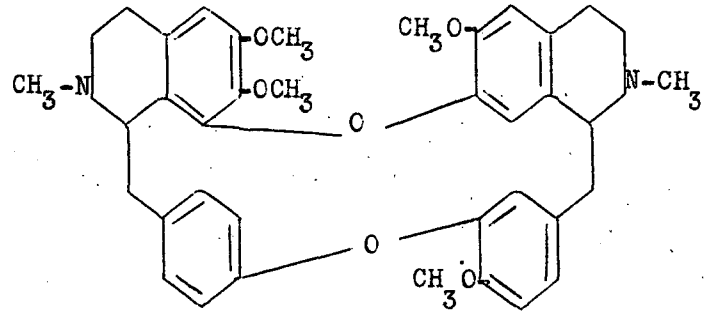
In the extractions carried out during the present investigation irregularities were also found in the alkaloid content of the other Daphnandra species. D. aromatica yielded only daphnoline while Bick and Whalley (87) report aromoline in this species as well. When this base failed to crystallise from the mother liquors left after the daphnoline crystallisations a portion of these liquors were subjected to partition chromatography between chloroform and hydrochloric acid in an effort to locate aromoline. However despite a thorough search no trace of this alkaloid was found. Again in the case of D. dielsii repandine was isolated in quite large amounts together with the yellow alkaloid repanduline and some minor bases, O-methyl-repandine, tenuipine and possibly repandinine. This was the first occasion that repandine had been isolated from D. dielsii - from all previous extractions repanduline was the only major base obtained. D. dielsii is very similar morphologically to D. repandula, which is known to contain repandine, and it is possible that in spite of the care taken by officers of the Queensland Forestry Department in making the collection, some of the latter species was included in the D. dielsii material ; however it seems more likely that this is

another example of alkaloid variation within the genus Daphnandra. It would seem from these irregularities that either the botany of the Daphnandras requires further attention or that for some reason or other the alkaloid content within the species of this genus is not constant.

It should also be recorded here that in addition to the crystalline bases isolated from D. micrantha and D. aromatica a large quantity of amorphous base was present. This appeared to be mainly non-phenolic or cryptophenolic in nature. Although partition chromatography between acid and chloroform and column chromatography on alumina could be employed to separate the mixture into fractions of different basic strength or adsorption power no crystalline compounds could be obtained from the mixture.

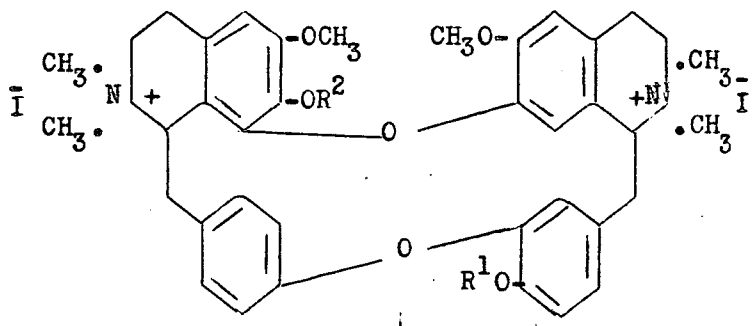
Bick, Ewens and Todd (28) have shown that daphnandrine, which contains one phenolic group and one secondary nitrogen atom, when methylated with sodium methoxide and methyl iodide yielded O,N-dimethyl-daphnandrine dimethiodide which was identical (X-ray powder diagram) with O-methyloxyacanthine dimethiodide ; the methine base obtained from O,N-dimethyldaphnandrine dimethiodide by a Hofmann degradation when heated with

methyl iodide yielded a crystalline methine dimethiodide which was identical with the methine dimethiodide obtained analogously from oxyacanthine. Hence it was evident that O,N-dimethyldaphnandrine was identical with O-methyloxyacanthine and hence both can be represented by (5.1). It follows from this that



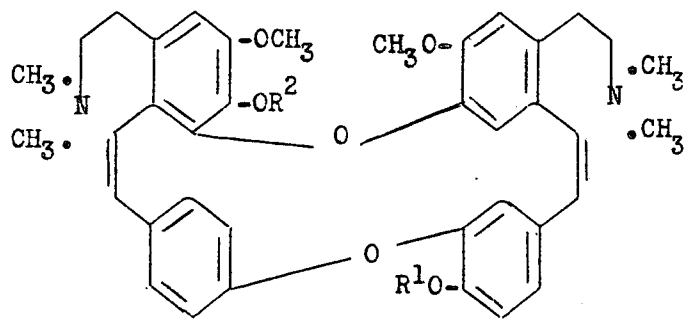
5.1

O,N-dimethyldaphnandrine dimethiodide must be (5.11, $R^1 = R^2 = CH_3$) and the methine derivable from this can be indicated by (5.111, $R^1 = R^2 = CH_3$). N-methyl, O-ethyl-daphnandrine dimethiodide which was obtained from the original base by methylation with methyl iodide followed by ethylation with ethyl iodide and sodium ethoxide gave on Hofmann degradation a methine base which on ozonolysis produced 2-methoxydiphenyl ether -5:4'-dialdehyde (5.1V, $R^1 = CH_3$). Hence it was obvious that the phenolic group in daphnandrine was situated in one of the tetrahydroisoquinoline nuclei and



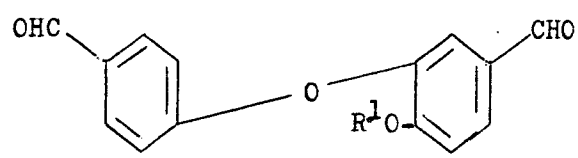
5.11

Hofmann degradation



5.111

ozone



5.1V

cannot occupy a position in one of the benzyl residues as it does in atherospermine or oxyacanthine.

Daphnoline (28) was shown to contain a secondary nitrogen group in addition to two phenolic groups one of which could be selectively methylated by diazomethane to yield daphnandrine. Thus daphnoline is a nordaphnandrine and in conformity with this it was shown by X-ray powder diagrams that O,O,N-trimethyldaphnoline dimethiodide and O,N-dimethyldaphnandrine dimethiodide were identical (5.11, $R^1 = R^2 = CH_3$). Daphnoline when methylated with methyl iodide followed by an ethylation with sodium ethoxide and ethyl iodide yielded N-methyl, O,O-diethyldaphnoline dimethiodide which when subjected to Hofmann degradation produced the corresponding methine base. Ozonolysis of this compound gave 2-ethoxydiphenyl ether -5:4'-dialdehyde (5.1V, $R^1 = C_2H_5$). Hence it is evident that one phenolic group in daphnoline is located at position 4 of a benzyl group and the other must occupy the same position as the phenolic group in daphnandrine.

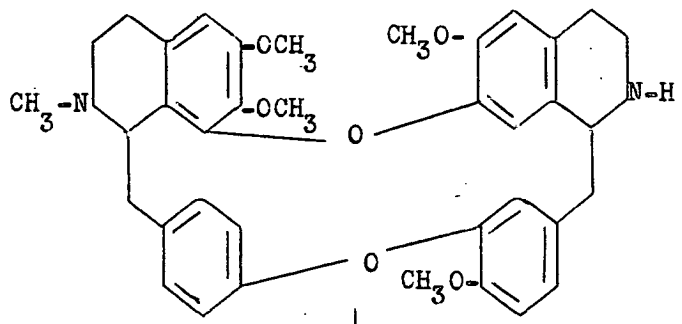
Aromoline which has two methylimino groups and two phenolic groups can be methylated to O,O-dimethyl-

aromoline dimethiodide the Debye-Scherrer diagram of which was shown to be identical with those of O-methyl-oxyacanthine dimethiodide and O,O,N-trimethyldaphnoline dimethiodide. Hence O,O-dimethylaromoline and O,O,N-trimethyldaphnoline can be also represented by (5.1). Aromoline dimethiodide when ethylated with ethyl iodide and sodium ethoxide gave O,O-diethylaromoline dimethiodide which by Hofmann degradation yielded a methine base which with methyl iodide yielded O,O-diethyl-aromoline methine dimethiodide. By X-ray powder diagrams it was shown that this dimethiodide was in fact identical with N-methyl, O,O-diethyldaphnoline methine dimethiodide. It follows therefore that aromoline is N-methyldaphnoline.

From the above preamble which is a summary of the work of Bick, Ewens and Todd (28) it follows that the structure of these three bases is complete with the exception of the positions of the hydroxyl group in daphnandrine, the second hydroxyl group in daphnoline and aromoline and the secondary nitrogen atom in daphnandrine and daphnoline. It is also obvious from the above account that since all three bases are so closely related structurally that if the complete

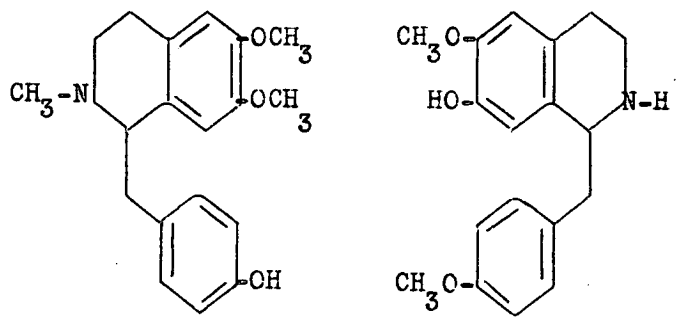
structure of one alkaloid could be determined the others would automatically follow. Since the present author had a reasonable quantity of daphnoline from the extraction of Daphnandra aromatica this base was selected for sodium in liquid ammonia reductions to help complete the information required to formulate completely this trio of alkaloids.

Methylation of daphnoline with diazomethane gave O,O-dimethyldaphnoline which when cleaved with sodium in liquid ammonia produced a mixture of phenols which was separated into its constituents by way of the oxalate salts. The addition of ethanolic oxalic acid to an ethanol solution of the crude phenols produced a crystalline precipitate of (+)armepavine oxalate from which (+)armepavine (5.V1) could be obtained in the usual way. The mother liquors remaining after the removal of this crystalline oxalate were made alkaline with ammonia and the basic material transferred to methanol in which solvent it was methylated with diazomethane. Purification of the O-methyl ether by chromatography gave a base whose properties were in accord with those of O,O-dimethylcoclaurine (5.V11). This series of reactions places the secondary nitrogen atom in daphnoline and hence O,O-dimethyldaphnoline



5.V

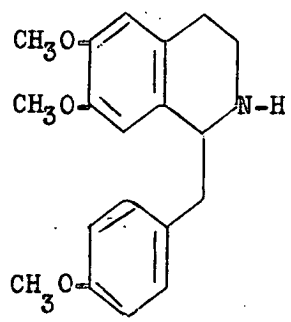
sodium
liquid ammonia



5.Vl

5.Vla

diazomethane



5.Vll

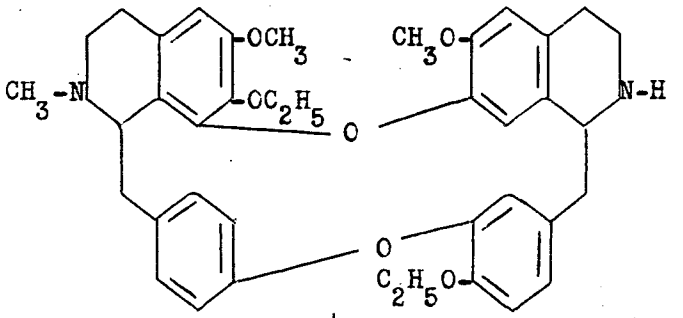
can be written as (5.V).

Daphnoline when ethylated with diazoethane produced 0,0-diethyldaphnoline which when reductively cleaved with sodium and ammonia produced a mixture of phenols which could not be conveniently separated. Ethylation of the whole produced the mixed 0-ethyl ethers which could be separated into two amorphous fractions by chromatography on alumina. Unfortunately however neither the bases themselves nor their methiodides could be obtained in a crystalline condition but some light was shed on the identity of the two cleavage products by consideration of the R_f values of the bases and their methiodides.

TABLE 5.2

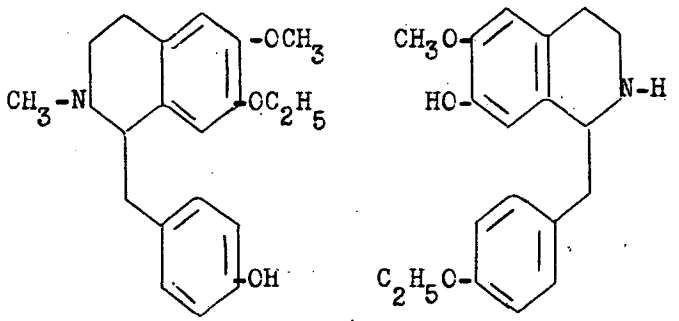
	R_f Base	R_f Methiodide
Fraction A	0.79	0.84
Fraction B	0.88	0.84

It is interesting to note in the above Table 5.2 that while the R_f values of the two ethylated degradation products are quite distinct the R_f values of their methiodides, in each of which the nitrogen atoms have been quaternised, are the same. This indicates that the structural difference between these two degradation products lies in their nitrogen atoms -



5.VIII

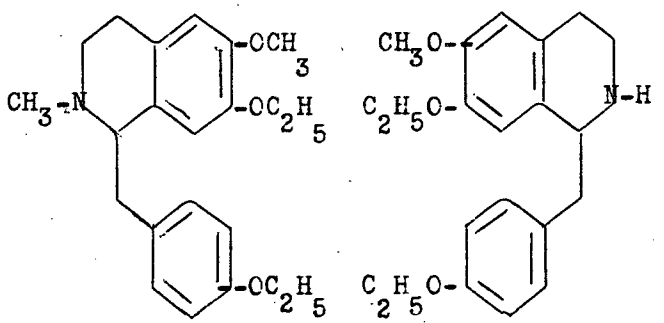
sodium
liquid ammonia



5.IX

5.X

diazoethane

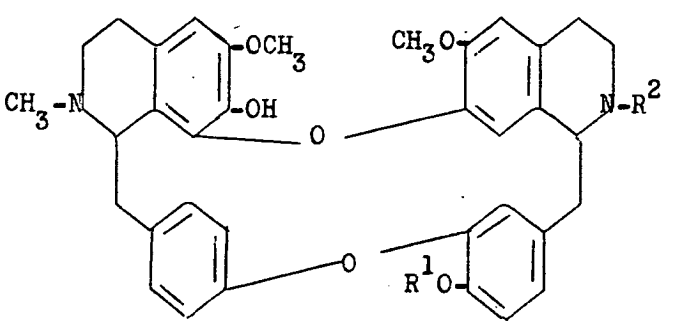


5.XI

5.XII

one being secondary and the other tertiary. Since the position of the methylimino group has been fixed in daphnoline by the cleavage of its O,O-dimethyl ether and since it is clear from Bick, Ewens and Todd's work (28) that daphnoline is a member of the oxyacanthine group of bases and has a phenolic group at position 4 of the benzyl unit it follows that from the fission of O,O-diethyldaphnoline one of the cleavage products must be (5.X) and this on ethylation would yield O,O-diethyl-coclaurine (5.X11). Now if the second ethylated degradation product is structurally identical with O,O-diethylcoclaurine with the exception that its nitrogen is tertiary then it must be N-methyl, O,O-diethyl-coclaurine (5.X1) and it follows that O,O-diethyl-daphnoline is represented by (5.V111).

If this reasoning is correct it follows that daphnoline can be represented by (5.X111, $R^1 = R^2 = H$)



5.X111

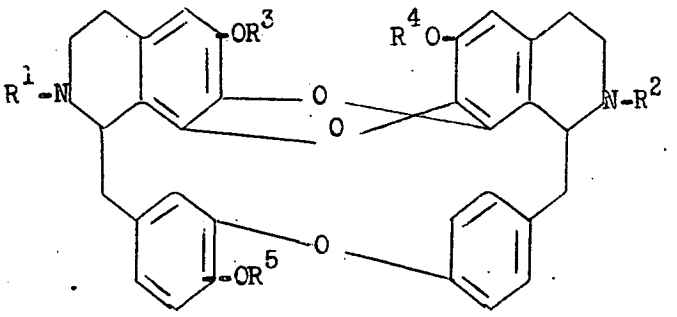
and from the structural relationships established by Todd et al. (28) it follows that aromoline must be (5.XIII, R^1= H, R^2= CH_3) and daphnandrine (5.XIII, R^1= CH_3, R^2= H). It is of interest to record that under this scheme daphnandrine has a hydroxyl group at the 7 position of the tetrahydroisoquinoline nucleus and as such should give a positive Millon's test according to King's observations (48, 60) ; daphnandrine in fact does give a pink colour with Millon's reagent.

Micranthine, another of the Daphnandra alkaloids, has been shown by Bick and Todd (77) to have a secondary nitrogen group and two phenolic groups ; an additional feature of this base is its diphenylene-dioxy system. Ethylation of micranthine dimethiodide with sodium ethoxide and ethyl iodide gave N-methyl, O,O-diethylmicranthine dimethiodide which when subjected to a Hofmann degradation gave the N-methyl, O,O-diethylmicranthine methine ; ozonolysis of the last compound gave 2-ethoxyldiphenyl ether -5:4'-dialdehyde (5.IV, R^1= C_2H_5). This indicates that one of the phenolic groups is situated at the 4 position of the benzyl residue of the molecule while the other is located in the tetrahydroisoquinoline nucleus. Thus in this base also there was uncertainty concerning the

position of one hydroxyl group ; the location of the secondary nitrogen group was unknown and there was some doubt of the exact position of the ether linkages which constituted the diphenylenedioxy system.

It was hoped that reductive degradations with sodium in liquid ammonia would help in the formulation of a complete structure for micranthine. O,O-diethyl-micranthine, prepared by ethylating the base with diazoethane, was cleaved with sodium in liquid ammonia in the usual way. The product obtained was phenolic in nature and failed to give a test for a diphenylene-dioxy group but it was felt that with three ether linkages involved one treatment with sodium in liquid ammonia was not sufficient for a complete reaction. Hence the phenolic reaction product was methylated with diazomethane with the intention of subjecting this mixture to a further treatment with sodium in liquid ammonia. However it was found that this methylated product was practically insoluble in benzene or toluene and the cleavage experiment could not be satisfactorily performed.

It is also quite possible that the reductive fission of the ether linkages in micranthine might yield a much more complex product than has been the case with the

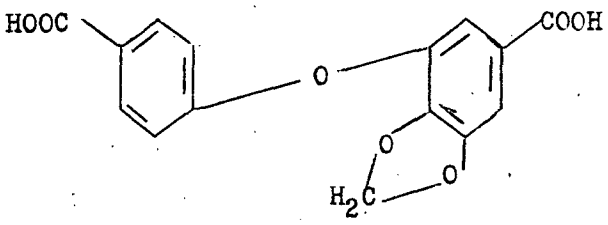


5.XIV

other bisbenzyl<u>iso</u>quinoline alkaloids. The present evidence from Bick and Todd's work (77) indicates that in all probability micranthine has a formula (5.XIV, R^5= H ; of R^1 and R^2 one is H and the other CH_3 ; of R^3 and R^4 one is H and the other CH_3). Hence O,O-dimethylmicranthine would be (5.XIV, R^3= R^4= R^5= CH_3 ; of R^1 and R^2 one would be H and the other CH_3). Thus in the tetrahydro<u>iso</u>quinoline half of the molecule there is an equal distribution of methoxyl groups about the diphenylenedioxy system. In all the other fully methylated alkaloids of this group (c.f. 5.V) this distribution of methoxyl groups about a particlar ether link has not been symmetrical. As pointed out by Birch (81) nucleophilic attack by sodium in liquid ammonia would result in cleavage of the ether link at

C_8 and would lead to the production of two coclaurine-type units such as (5.V1 and 5.V1a). This is borne out by the fact that in all previously mentioned fission experiments the ether linkages are always cleaved at the bond joining the oxygen to the benzyl*iso*quinoline unit carrying the greater number of alkoxyl groups adjacent to the bond in question. But in the case of micranthine it is doubtful if the cleavage would proceed to give only two coclaurine units ; it seems probable in fact that the mixture of products might well be rather complex.

Repanduline is the only major Daphnandra alkaloid about which little is known. It is a yellow base occurring in a number of Daphnandra species and is known to be non-phenolic and contain at least one methylene-dioxy group. Repanduline has not proved amenable to Hofmann or Emde degradations and is in fact generally rather unstable compared with other Daphnandra alkaloids. Bick, Doebel, Taylor and Todd (80) showed that this base belonged to the bisbenzyl*iso*quinoline group by oxidising it with potassium permanganate to repandulinic acid, a substance which by synthesis has been identified as 5:4'-dicarboxyl -2:3-methylenedioxy-diphenyl ether (5.XV). However this was the only

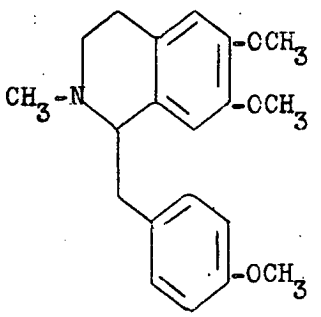


5.XV

degradation product which could be isolated and the chemistry of the tetrahydroisoquinoline portion of the repanduline molecule remained obscure. In fact it has been a very difficult matter to assign a molecular formula to this alkaloid ; repanduline holds solvent of crystallisation most tenaciously and this has resulted in inconsistent analytical figures. Bick and Whalley (27, 86) reported $C_{40}\ H_{46}\ O_8\ N_2$ for this base with two methoxyl and two methylimino groups. Todd et al. (80) gave a provisional formula $C_{37}\ H_{34}\ O_7\ N_2$ with one methoxyl and two methylimino groups. The repanduline which was analysed during the present investigation was crystallised from ether and dried at 50° under high vacuum conditions. Hence it is felt unlikely that the resultant repanduline contained solvent of crystallisation. The analytical figures returned on this sample were in remarkably good agreement (see experimental section) for $C_{36}\ H_{40}\ O_7\ N_2$ with two methoxyl groups present.

Sodium in liquid ammonia reductions on repanduline were complicated by the presence of the methylenedioxy group. This group is less stable to these reagents than the diphenyl ether linkages so is split first with the production of a phenolic group (94). Hence it was necessary to methylate the phenolic products obtained and submit the mixture to a second sodium-liquid ammonia treatment to ensure that all the diphenyl ether linkages present had been completely cleaved. However a small non-phenolic fraction was obtained from the first sodium-liquid ammonia fission and from this a small quantity of a colourless compound was separated from the unreacted repanduline. This fission product, which did not contain a methylenedioxy group (Labat test) analysed for $C_{20}H_{25}O_3N$ and contained two methoxyl groups, but the nature of the third oxygen could not be ascertained on the small amount of material available. This confirmed the presence of at least two methoxyl groups in the original repanduline for it is hardly possible for such a group to arise during the fission. As a methylene-dioxy group yields a phenolic unit on reduction with sodium in liquid ammonia this present reduction product, for convenience designated compound A, must be derived

from the benzyl<u>iso</u>quinoline half of the molecule which does not contain the methylenedioxy group. It is interesting to note that compound A is isomeric with O-methylarmepavine (5.XV1) and this fact makes it

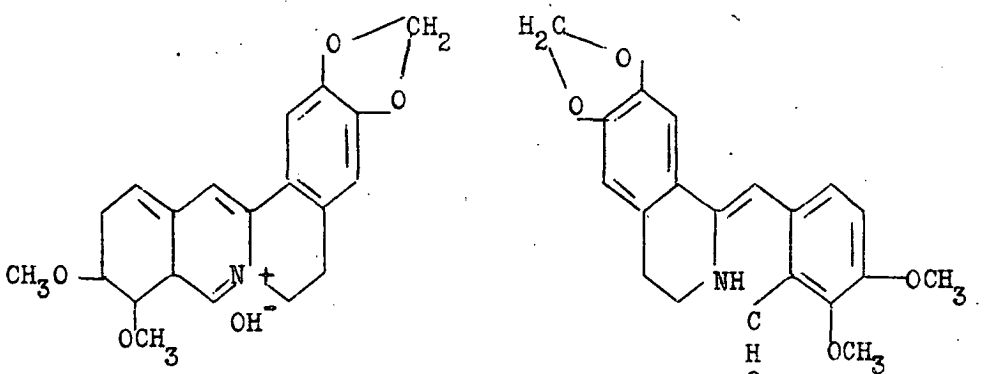


5.XV1

likely that the half of the repanduline molecule from which it is derived is not very different from a normal benzyl<u>iso</u>quinoline unit. However there must be at least one modification for in O-methylarmepavine the three oxygen atoms are involved in methoxyl groups while in compound A only two methoxyl groups are present. If it is assumed that compound A has a normal benzyl<u>iso</u>quinoline skeleton with two methoxyl groups present the molecular formula then requires an extra CH_2O. One possibility is tc formulate the compound with a primary alcohol group and this also is one of the few neutral oxygen-containing groups likely to be present in a product of sodium in liquid ammonia

reduction. Such a primary alcohol group was not found by Todd et al. (80) in the original repanduline so if present in the compound A it must have been formed during the cleavage, either by the reduction of an aldehyde or by the fission of a benzyl ether.

A crypto-aldehyde group such as that in berberine (5.XX) would be expected to yield a primary alcohol

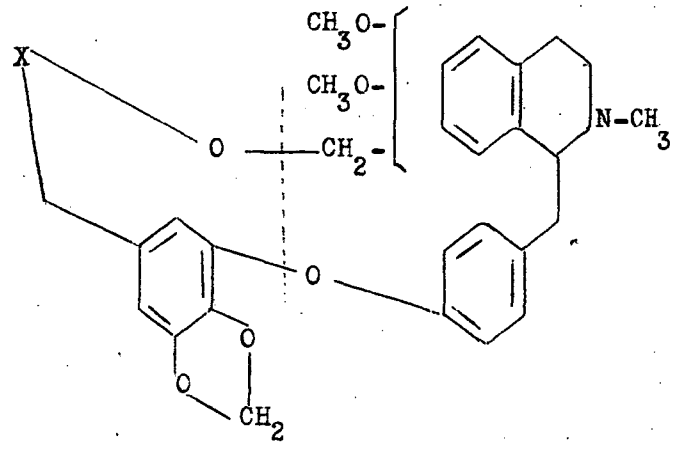


5.XX

on sodium-ammonia reduction and this might also explain the yellow colour of repanduline on similar lines to that of berberine ; however no evidence for such a group could be found by Todd et al. (80) in repanduline and moreover the primary alcohol group in the reduction product would have to be located in the benzyl residue, which would not be in accord with the isolation of repandulinic acid as an oxidation product of repanduline.

If the second alternative is correct then a partial

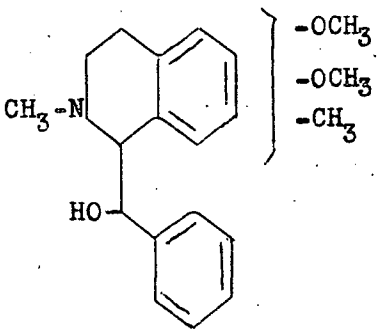
formula (5.XV11) might be written for repanduline.



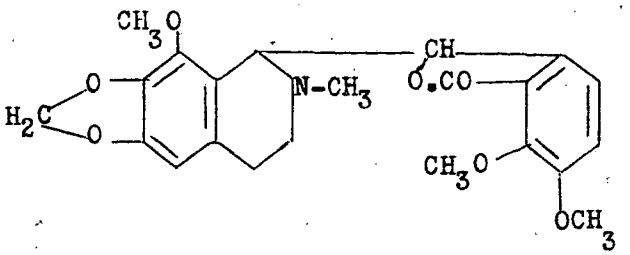
5.XV11

It should be pointed out however that such a partial formula would be expected to cleave in the positions indicated which would yield a phenolic group in the benzyl unit and a methyl group in the <u>iso</u>quinoline nucleus. However since only a small yield (6%) of compound A is obtained it might well be a by-product of the main reaction ; also the group X might well affect the cleavage, as for instance in the case of a dibenzyl ether type of link.

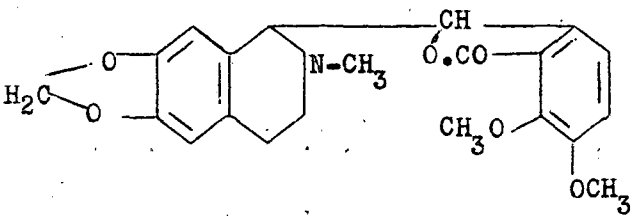
Another possible formulation for compound A has the oxygen as a secondary alcohol group as shown (5.XV111) ; a number of well known benzyl<u>iso</u>quinoline alkaloids such as narcotine (5.XX1) and hydrastine (5.XX11) are oxygenated in this position and in this case the



5.XVIII



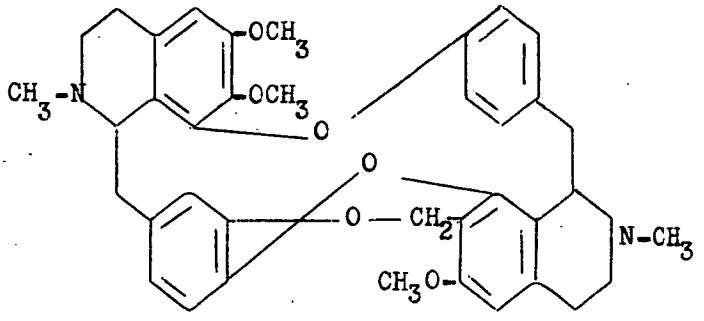
5.XXI



5.XXII

remaining carbon atom would be present as a methyl group, formed possibly by reductive fission of a benzyl ether. An example of a bisbenzyl<u>iso</u>quinoline alkaloid containing a linkage of this kind is insularine (5.X1X) the chemistry of which was investigated by Kondo, Tomita and Uyeo (95, 98, 99, 100, 101). However the reduction of this base with sodium in liquid ammonia has not as yet been attempted.

The phenolic fraction which was the major product from the first sodium in liquid ammonia fission on



5.XIX

repanduline was methylated and subjected once again to the cleavage. Although the reaction proceeded farther as indicated by the appearance of another phenolic fraction no crystalline compound has as yet been identified from the second fission.

It might also be mentioned that fission experiments with repanduline do not proceed as smoothly as with other bisbenzyl<u>iso</u>quinoline bases ; the yields are lower (<u>ca</u> 75%) while other bases of this group cleave in an almost quantitative fashion. Also a large amount of a brown amorphous matter is obtained from repanduline during the reaction. It has been reported by Bick (96) that such amorphous material always accompanies any reaction with repanduline.

Further evidence is necessary to confirm or disprove the above speculations concerning the structure

of compound A, but despite the low yield in which it is obtained, which suggests that it is a by-product in the reduction, a knowledge of its structure would undoubtedly be of great assistance in the understanding of the various problems associated with the structure of repanduline.

EXPERIMENTAL

Alkaloids of Daphnandra micrantha. Sundried bark (5 Kg.) was ground and moistened with lime (250 g.) and dried again in a vacuum oven. Extraction with methanol in a Soxhlet type apparatus followed and this was continued until the extracts gave only slight Meyer's tests (nine days ; the bark was mixed thoroughly every 2-3 days). The methanol solution was evaporated at normal pressure to a volume of 15 litres when it was made acid with aqueous tartaric acid. A copious crystalline precipitate of potassium hydrogen tartrate formed immediately and after allowing this to stand in a refrigerator overnight it was removed by filtration. The insoluble tartrate was repeatedly extracted with boiling water to remove any adhering alkaloid and the aqueous washings were added to the main extract and the whole evaporated to a volume of about 5 litres. From time to time during this evaporation further amounts of potassium hydrogen tartrate precipitated and after being well washed were rejected. The aqueous tartaric acid solution which contained all the basic material extracted from the bark was made alkaline with ammonia and the precipitated alkaloids were removed by filtration and dried in a

vacuum oven. The dried crude alkaloids were extracted with chloroform - first by cold percolation and finally by the boiling solvent. At the end of this procedure a resinous material remained which, while still giving strong Meyer's tests, proved difficult to dissolve in chloroform. This gum was extracted with acetic acid (5%), the solution was filtered and the clear filtrate made alkaline with ammonia ; the alkaloids precipitated in this way were removed by filtration, dried and extracted in a Soxhlet with chloroform. All chloroform extracts were joined and evaporated to a conveniently small volume (1.5 litres). The chloroform extract was extracted in turn with 5% sodium hydroxide and 1% sodium hydroxide to remove the strong and weak phenols respectively. The chloroform solution at this stage contained the cryptophenols and non-phenols.

The 5% sodium hydroxide extract was made acid with hydrochloric acid and the solution filtered to remove non-basic material. The acid filtrate was basified with ammonia and the small amount of precipitate extracted with chloroform. Removal of the solvent, after drying, left a small residue (ca 1 g.) which refused to crystallise and gave no indication of containing daphnoline.

The 1% sodium hydroxide extract was acidified with hydrochloric acid and filtered to remove resinous material insoluble in acid. This was washed well with dilute hydrochloric acid (1%) and rejected. The acid extract was basified with ammonia and the copious precipitate removed by chloroform. After drying the latter solution over sodium sulphate the solvent was reduced in volume *in vacuo* and the solution set aside when micranthine (60 g.) crystallised.

The chloroform solution which remained following the alkaline extraction was thoroughly washed with water and dried over sodium sulphate. Evaporation of chloroform left the cryptophenolic and non-phenolic bases in the form of a gummy residue (*ca* 100 g.) which gave very strong alkaloid tests in addition to producing a blue coloration with sulphuric acid containing a trace of nitric acid. This latter reaction is characteristic of the phenylenedioxy system (74, 75, 76). This residue was divided into three portions by its solubility in the following solvents - benzene (*ca* 60 g.), chloroform : benzene (1:3 ; *ca* 30 g.) and chloroform (*ca* 10 g.). Each of these portions were then chromatographed in the solvents used to dissolve them. By the use of a fraction-cutter the first two portions

could be subdivided into small groups but all refused to crystallise from the usual solvents. A counter-current distribution of samples from the benzene and benzene:chloroform (1:3) portions between chloroform and dilute aqueous hydrochloric acid of varying strength again failed to produce any crystalline product. The chloroform soluble portion after elution from an alumina column with methanol:chloroform (1:9) crystallised from chloroform to yield a further crop of micranthine (5 g.). The amorphous bases were set aside for examination at a later date.

Alkaloids of Daphnandra aromatica. Sundried bark of Daphnandra aromatica (6.5 Kg.) collected in Queensland was extracted in a Soxhlet (five days). After acidification with aqueous tartaric acid the extract was evaporated under reduced pressure to remove the methanol ; the extract was then diluted with water which increased the volume (10 litres) and also served to precipitate a quantity of non-basic material which was removed by filtration, washed thoroughly with dilute tartaric acid and rejected. Aqueous ammonia was added to the acidic extract until the pH was about 11 ; the precipitated bases were removed by filtration and dried in a vacuum oven. The crude base (220 g.) was extracted exhaustively with chloroform in a Soxhlet and

the resultant solution was extracted with sodium hydroxide (5%) until all the phenolic bases had been removed.

The resultant aqueous alkaline solution was acidified with hydrochloric acid and the precipiated non-basic material was again rejected after a thorough washing with dilute acid. The bases were precipitated from the acid solution with ammonia and transferred to chloroform. This latter solution after drying (sodium sulphate) and reduction in volume slowly deposited daphnoline (6.5 g.). A thorough search of the mother liquors which accumulated from the daphnoline re-crystallisations failed to reveal any evidence of the presence of aromoline.

The chloroform solution which remained after the extraction of the phenolic bases was washed thoroughly with water and dried over sodium sulphate. Removal of the solvent <u>in vacuo</u> left a residue (50 g.) which although giving very strong Meyer's tests could not be separated into crystalline bases either by chromatography on alumina or by partition on a counter-current machine between chloroform and aqueous hydrochloric acid of increasing strength. These amorphous bases were also set aside for future examination.

Alkaloids of Daphnandra dielsii. Dried ground bark (7 Kg.) from Daphnandra dielsii was extracted with benzene in a Soxhlet until the extract gave only weak Meyer's tests (nine days). At this stage the solution was evaporated to a small volume (1 litre) and an equal volume of methanol added ; the mixture was then set aside in a refrigerator. Overnight some crude crystalline base (10 g.) settled out which was removed from the extract by filtration. The filtrate was evaporated under reduced pressure to produce a gummy residue which was exhaustively treated with hydrochloric acid (1% ; 6 x 500 c.c.) and the non-basic residue removed from the acid extract by filtration. The filtrate was made alkaline with ammonia and the copious precipitate transferred to a chloroform solution by gentle agitation with this solvent (3 x 500 c.c.). Extraction of the chloroform solution with sodium hydroxide (5% ; 3 x 500 c.c.) removed phenolic material (less than 1 g. which although yielding a strong Meyer's test failed to crystallise from the normal solvents) and after washing with water the chloroform was dried over sodium sulphate. The solvent was removed under reduced pressure and the residue taken up in benzene. This latter solution was chromatographed on alumina and the column washed with the following solvents.

Benzene		no material removed
50% Benzene ...	50% Chloroform	a fraction-cutter used to collect the washings separated the bases into two groups
	(1) a colourless fraction	eluate A
	(2) a yellow fraction	eluate B
Chloroform		eluate C

The semicrystalline mass which was separated from the benzene-methanol solution mentioned above, was dissolved in hydrochloric acid (1%) and after filtration the basic material was precipitated by the addition of aqueous ammonia. Chloroform (500 c.c.) was used to dissolve the suspended alkaloid and this solution after a wash with sodium hydroxide (2 x 250 c.c.) followed by water was dried over sodium sulphate and the solvent replaced with benzene. Chromatography on alumina followed as described above to yield further amounts of eluates A, B, C.

Eluate C. Removal of the chloroform left a white amorphous solid which dissolved in boiling ethanol to produce fine needles of repandine (5.1 g.).

Eluate B. Removal of the chloroform-benzene left a yellow semi-solid residue which crystallised readily when a warm methanol solution of this base was allowed to cool producing yellow needles of repanduline

(21.5 g.).

Eluate A. The colourless residue remaining when the solvent was removed refused to crystallise so was redissolved in benzene and rechromatographed collecting smaller fractions than on the previous occasion ; as a result two crystalline bases were separated - tenuipine and O-methylrepandine.

Alkaloids of Daphnandra tenuipes. Sundried bark (1.7 Kg.) from Daphnandra tenuipes was milled and extracted with benzene and the extract chromatographed on alumina. Elution with benzene-chloroform (1:1) produced a colourless eluate (D) and a yellow eluate (E).

Eluate D. Removal of the solvent left a colourless residue which crystallised from methanol to give tenuipine (0.5 g.) as colourless prisms.

Eluate E. Removal of the solvent left a yellow solid which crystallised from methanol to give repanduline (1 g.) as pale yellow needles.

No attempt was made to separate the phenolic bases from D. tenuipes which explains the non-appearance of aromoline.

Micranthine. Micranthine recrystallised repeatedly from chloroform and methanol had m.p. 194-5° (sinters 185°) (undepressed by an authentic sample of this base

obtained by Bick and Todd (77) from D. micrantha) and $[\alpha]_D^{21} = -230$ (c,0.5 in $CHCl_3$ in 4 dcm. tube). Bick and Todd (77) give m.p. 194-6° and $[\alpha]_D^{22} = -231$ ($CHCl_3$) as the physical constants of this base. With concentrated sulphuric acid containing a trace of nitric acid micranthine gave a deep blue colour characteristic of compounds containing the phenylene-dioxy system ; a ferric chloride test proved negative and the Millon's reagent gave a faint pink colour. These colour reactions are in accordance with the findings of previous workers.

Found

C,69.9 H,6.2 O,19.0 NCH_3,5.1 CH_3O,5.3%

Calc. for $C_{34}H_{32}O_6N_2.H_2O$

C,70.1 H,5.9 O,19.2 1NCH_3,5.0 1CH_3O,5.3%

Daphnoline. Daphnoline repeatedly recrystallised from chloroform and methanol had m.p. 190-200° (not sharp) and $[\alpha]_D^{18.5}$ +438 (c, 0.3 in $CHCl_3$ in 4 dcm. tube) (rotation calculated for free base). With ferric chloride a greenish colour was produced and the base gave a positive Millon's test. Bick and Whalley (78) report m.p. 194-5° and $[\alpha]_D$ = +441 ($CHCl_3$ calculated for free base) for this base but Pyman (79) in his original paper on daphnoline reports a wide m.p. range

190-215°.

Found

C,71.3 H,6.3 CH_3O,10.3%

Calc. for $C_{35}H_{36}O_6N_2.\frac{1}{2}H_2O$

C,71.0 H,6.55 $2CH_3O$,10.5%

Repanduline. Repanduline, purified by recrystallisation from chloroform/methanol, methanol and ether to give pale yellow needles, had $[\alpha]_D^{18.5}$= +470 (c, 0.4 in $CHCl_3$ in 4 dcm. tube). This base shrinks to a dark gum at 175° and slowly decomposes as the temperature is raised. The Millon's test with repanduline gave a negative result but the Labat test (73) for a methylene-dioxy group was positive. Bick, Doebel, Taylor and Todd (80) give $[\alpha]_D^{17}$= +473 ($CHCl_3$) and their report also indicates the same colour reactions of repanduline and reaction to heat as mentioned above.

Found

C,70.6 H,6.6 O,17.7 N,4.6 CH_3O,10.2%

Calc. for $C_{36}H_{40}O_7N_2$

C,70.6 H,6.6 O,18.3 N,4.5 $2CH_3O$,10.1%

Repandine. Repandine recrystallised from ethanol, methanol or acetone tc give fine colourless needles,

had $[\alpha]_D^{20} = -106$ (c, 0.5 in $CHCl_3$ in 4 dcm. tube) and m.p. 254° undepressed by an authentic sample of this base isolated by Bick and Todd (21) who report m.p. 255° and $[\alpha]_D^{15} = -106$ ($CHCl_3$) for this compound. The Millon's test was positive which is characteristic of repandine.

Found

C,71.4 H,6.7 O,16.6 N,4.2 CH_3O,15.5%

Calc. for $C_{37}H_{40}O_6N_2 \cdot \frac{1}{2}H_2O$

C,71.9 H,6.7 O,16.9 N,4.5 $3CH_3O$,15.1%

<u>O-Methylrepandine</u>. O-Methylrepandine recrystallised from methanol as needles, had $[\alpha]_D^{18} = -74$ (c, 0.2 in $CHCl_3$ in 4 dcm. tube) and m.p. 211° alone or mixed with authentic O-methylrepandine isolated by Bick, Taylor and Todd (33). These authors give m.p. 211° and $[\alpha]_D = -73$ ($CHCl_3$) as the physical constants for this base. O-Methylrepandine gave a negative Millon's test.

Found

C,73.3 H,6.8%

Calc. for $C_{38}H_{42}O_6N_2$

C,73.3 H,6.8%

Tenuipine. Tenuipine when recrystallised repeatedly from methanol gave colourless prisms which had $[\alpha]_D^{18} = -256$ (c, 0.5 in $CHCl_3$ in 4 dcm. tube) and m.p. 143° which was undepressed by an authentic sample of tenuipine isolated by Todd et al. (33) from Daphnandra tenuipes. In their report these authors record the following for the physical constants of this base; m.p. 143-145° and $[\alpha]_D^{20} = -258$ ($CHCl_3$). Tenuipine gave a positive Labat test for a methylenedioxy group and produced a pink coloration with concentrated sulphuric acid. The Millon's test was negative. These observations were in accord with the findings of Todd and his co-workers (33).

Found

C,71.4 H,6.3 O,17.8 N,4.2 CH_3O,14.7%

Calc. for $C_{38}H_{40}O_7N_2$

C,71.7 H,6.3 O,17.6 N,4.4 $3CH_3O$,14.6%

Methylation of Daphnoline. Daphnoline (0.8 g.) was dissolved in methanol (150 c.c.) and methylated with diazomethane in ether (from 3 g. of methylnitrosourea) for a period of a fortnight. During this time four such additions of ethereal diazomethane were made. The oil which remained following the removal of the solvents

under reduced pressure was dissolved in benzene and purified by chromatography on an alumina column from which it could be eluted with benzene-chloroform (1:1). The 0,0-dimethyldaphnoline (0.4 g.) so purified would not crystallise from the usual solvents but it was shown to be non-phenolic in character by its insolubility in Claisen's reagent (methanolic potassium hydroxide) which dissolves cryptophenolic compounds.

Fission of 0,0-Dimethyldaphnoline. 0,0-Dimethyldaphnoline (0.3 g.) was dissolved in toluene (30 c.c.) and was cleaved with sodium (1 g. in all) in liquid ammonia (400 c.c.). After the evaporation of the ammonia the non-phenolic products (0.03 g.) were separated from the phenolic ones (0.25 g.) by the usual means and the latter were dissolved in ethanol (2 c.c.) and a saturated solution of oxalic acid in ethanol (2 c.c.) was added and the whole set aside in a refrigerator overnight. The insoluble crystalline oxalate (0.1 g.) which settled out during this time was purified by repeated recrystallisation from ethanol when it had m.p. 209° both on its own and in mixture with (+)armepavine oxalate. Tomita (10) reports 212° as the m.p. of this salt.

Found

C,62.2 H,6.8 O,27.4 NCH_3,5.9%

Calc. for $C_{19}H_{23}O_3N.\frac{1}{2}(C_2O_4H_2).1\frac{1}{2}H_2O$

C,62.3 H,7.1 O,27.0 NCH_3,7.5%

Dissolution of the oxalate in warm water followed by the addition of ammonia liberated the free base which was extracted with ether and the resultant solution dried (sodium sulphate). Removal of the solvent left armepavine as a gum which crystallised from ether/acetone to have m.p. 143° compared with m.p. 145° reported by Tomita (10).

The mother liquors remaining after the removal of the crystalline oxalate were evaporated to dryness under reduced pressure and the residue taken up in warm water. The aqueous solution was made alkaline with ammonia and the basic material removed by ether extraction. After drying (sodium sulphate) and removal of solvent the residue (0.11 g.) was dissolved in methanol and methylated with ethereal diazomethane (1 g. of methyl-nitrosourea). Two further such additions of ethereal diazomethane were made at three day intervals ; the solvent and excess diazomethane were removed <u>in vacuo</u> and the residue was dissolved in benzene and purified

by chromatography. The base was eluted with benzene-chloroform (1:1) and the residue (0.05 g.) which remained following the removal of the eluting solvent was obtained in a crystalline state when moistened with alcohol. Recrystallisation gave 0,0-dimethyl-coclaurine m.p. 200°. Tomita (6) reports 202-3° for this base.

Ethylation of Daphnoline. Daphnoline (1.0 g.) was dissolved in methanol (200 c.c.) and ethylated with ethereal diazoethane as described above for the methylation of this base. As a result 0,0-diethyl-daphnoline (0.7 g.) was obtained as an oil which again would not crystallise even following a chromatographic purification. However the complete ethylation of the compound was shown by its insolubility in Claisen's reagent.

Fission of 0,0-Diethyldaphnoline. 0,0-Diethyldaphnoline (0.6 g.) was cleaved with sodium (1 g. added piece-wise) in liquid ammonia (400 c.c.) to yield a mixture of phenols (0.58 g.) which was separated from the small amount of non-phenolic starting material in the usual way. The phenols were dissolved in methanol (30 c.c.) and ethylated with ethereal diazoethane during a period of ten days. Removal of the solvent and excess diazoethane left a residue which was

dissolved in benzene and chromatographed on alumina. Elution with benzene washed a yellow oil (0.24 g.)from the column (degradation product A) while elution with chloroform : benzene (1:1) yielded another oil (0.22 g.) (degradation product B). Attempts to crystallise these fractions or their methiodides failed.

Paper Chromatography of the Ethylated Fission Products of O,O-Diethyldaphnoline. The R_f values of the two ethylated fission products of O,O-diethyldaphnoline together with their methiodides were determined on Whatman No. 1 paper using butanol :-acetic acid : water (4:1:5 ; top layer) as solvent for a period of 16 hours. The spots were developed with iodine vapour. The results have been recorded in Table 5.2.

Fission of Repanduline. Repanduline (1.6 g.) was dissolved in toluene-benzene (30 c.c. : 10 c.c.) and cleaved with sodium (1.4 g. added piece-wise) in liquid ammonia (500 c.c.). The phenolic fraction (0.82 g.) was separated from the non-phenolic fraction (0.21 g.) in the usual way.

Non-phenolic Fraction. The non-phenolic fraction was dissolved in benzene and chromatographed on alumina. Elution with the following solvents gave these results.

Benzene	eluate A
75% Benzene ... 25% Chloroform	no material removed
50% Benzene ... 50% Chloroform	eluate B

Eluate B. Removal of the eluting solvent left a yellow gum which crystallised readily from methanol to give repanduline (0.10 g.) m.p. 175-185°d., $[\alpha]_D^{18} = +471$ (c, 0.2 in $CHCl_3$ in 4 dcm. tube). These physical constants are in accord with those reported for repanduline.

Eluate A. Removal of the benzene left a residue which crystallised readily from ether/petroleum ether (40-60°) to yield needles m.p. 147°. This compound gave a strong Meyer's test but yielded negative results for a methylenedioxy group (Labat test).

Found

C,73.2 H,7.7 O,15.0 N,4.5 CH_3O,18.9%

Calc. for $C_{20}H_{25}O_3N$

C,73.4 H,7.7 O,14.7 N,4.3 $2CH_3O$,19.0%

Phenolic Fraction. The phenolic fraction (0.82 g.) was dissolved in methanol (20 c.c.) and methylated with ethereal diazomethane over a period of a week. The non-phenolic product after separation from any unreacted phenolic material was dissolved in a benzene-toluene (10 c.c. : 20 c.c.) mixture and subjected again to a

reductive fission with sodium (1 g.) in liquid ammonia (400 c.c.). The reaction mixture remaining after the evaporation of the ammonia was separated by the usual methods into a phenolic (0.22 g.) and a non-phenolic (0.6 g.) fraction both of which have failed to date to yield crystalline compounds.

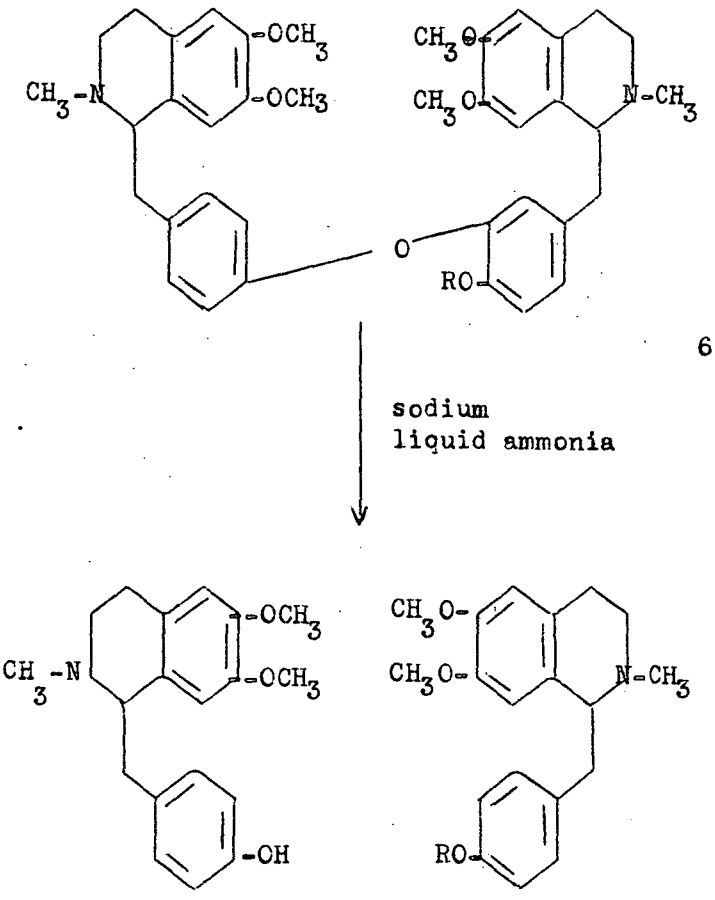
PART 6

FISSION OF DAURICINE WITH SODIUM IN LIQUID AMMONIA

Dauricine was first isolated by Kondo and his collaborators (42) from the Far Eastern plant Menispermum dauricum. Degradation experiments by these workers revealed that this alkaloids was a bisbenzylisoquinoline base having the formula (6.1, R= H).

Some years ago Manske in Canada isolated from Menispermum canadense (43) a base which yielded the same degradation products as the Japanese dauricine and resembled this base in its physical constants. Due to the recent war a direct comparison of the two alkaloids was impossible although there seemed little doubt of their identity. By the kindness of Dr. Manske a sample of his base was made available to the present author and after methylation and reduction with sodium and liquid ammonia a phenolic and non-phenolic product were obtained from the reaction mixture. These were separated in the usual way and shown to be (-)armepavine (6.11) and (-)O-methylarmepavine (6.111, R= CH_3) respectively.

During the course of the present investigation there appeared a report (44) of some Japanese work



6.1

6.11

6.111

on dauricine isolated in Japan. This report indicated that by using a similar reductive fission the Japanese dauricine could be cleaved to exactly the same products as mentioned above. The coincidence of these findings further supports the identity of the two dauricines obtained from the different species and also reveals that both asymmetric centres in this base are of the (-) type.

EXPERIMENTAL

O-Methyldauricine. Dauricine (0.6 g.) dissolved in methanol (50 c.c.) was methylated with diazomethane following procedures already described. The O-methyldauricine so obtained refused to crystallise as did a small sample converted to its dimethiodide.

Fission of O-Methyldauricine. O-Methyldauricine (0.4 g.) was dissolved in toluene (30 c.c.) and cleaved with sodium (1.2 g.) in liquid ammonia (400 c.c.). The non-phenolic and the phenolic fractions were separated and purified by the usual methods. The non-phenolic base was warmed with methanolic methyl iodide to convert it to its methiodide, m.p. 135°, $[\alpha]_D^{18} = -118$ (c, 0.2 in CH_3OH). These findings indicated that the methiodide was (-)O-methylarmepavine methiodide and this was confirmed by a mixed m.p. determination with an authentic sample which produced no depression ; a marked lowering of the m.p. occurred however with (+)O-methyl-armepavine methiodide.

Found

C,51.2 H,6.5%

Calc. for $C_{20}H_{25}O_3N.CH_3I.1\frac{1}{2}H_2O$

C,51.3 H,6.3%

The phenolic base was dissolved in ethanol (2 c.c.) and a saturated ethanolic solution of oxalic acid added (2 c.c.). After some time a crystalline mass settled out which on repeated recrystallisation from ethanol yielded (-)armepavine oxalate m.p. 209°, $[\alpha]_D^{18}$ = -81 (c, 0.3 in H_2O). Tomita (10) reports 212° for the m.p. of this oxalate. Solution of the salt in warm water followed by the addition of ammonia precipitated the free base which was extracted with ether. After drying and removal of the solvent armepavine was obtained as a crystalline base m.p. 144°. Tomita (10) gives 145° for the m.p. of this base. Methylation of a methanolic solution of armepavine with diazomethane gave the O-methyl ether which was identified as usual as its methiodide, m.p. 135° and $[\alpha]_D^{18}$= -117 (c, 0.2 in CH_3OH). The m.p. of this compound was undepressed on admixture with (-)O-methylarmepavine methiodide but a depression of over 10° occurred when it was melted in mixture with (+)O-methylarmepavine methiodide.

Found C,50.8 H,6.4%

Calc. for $C_{20}H_{25}O_3N.CH_3I.1\frac{1}{2}H_2O$ C,50.8 H,6.3%

APPENDICES

APPENDIX A

A PRELIMINARY INVESTIGATION OF THE OCCURRENCE OF ALKALOIDS IN THE TASMANIAN FLORA

At the conclusion of the recent war it was decided to undertake a phytochemical survey of Australia principally with a view to find plants which might yield natural products of medicinal importance. Collection services were arranged, laboratory facilities made available and some clinical tests carried out. In the relatively few years that have elapsed since the conception of this scheme a great many plants have been investigated. In particular, those bearing alkaloids have received the most attention - probably because spot tests lend themselves to the identification of this group of chemical compounds. Webb's survey of alkaloid-bearing species in Queensland deserves special mention. The result of his expeditions have been the subject of two C.S.I.R.O. Bulletins (35, 36). Plant material was collected and extracted in the field with either 1% hydrochloric acid or Prollius solution (37) and these extracts were tested with the usual alkaloid reagents. The amount of precipitate was used to give a guide to the basic content of the plant. His

results have been most useful to Australian chemists in deciding which particular species should be given prior attention.

In Tasmania it was decided to investigate the local flora, following Webb's experimental method, to ascertain the species bearing alkaloids. The results of such tests are summarised in Table A.1.

Experimental Methods.

Small samples (2-3 g.) of plant material were soaked in 1% hydrochloric acid or Prollius solution (37) for twenty-four hours. After filtration (and in the case of the Prollius reagent removal of organic solvents and extraction of residue with 1% hydrochloric acid) a drop of an alkaloidal precipitating reagent was added to 1-2 c.c. of the acid solution. The reagents used, in the main, consisted of Meyer's solution [mercuric chloride, potassium iodide (38)], Wagner's solution [potassium iodide, iodine (39)] and saturated aqueous picric acid. Following Webb's practice a quantitative estimation of the alkaloid content was indicated. A very heavy precipitate was indicated by ++++ while a slight turbidity was represented by a single +. Intermediate amounts were indicated by +++ or ++ depending on the quantity of material precipitated.

Discussion of Results.

During the course of this investigation sixty-one species have been examined fifteen of which were endemic in this state. Although this represents only a very small percentage of Tasmania's flora it is a fair cross section of the plant population. Hence it would seem reasonable to deduce that this State is not rich in alkaloid-bearing plants. This might have been expected from geographical considerations for it is well known that tropical and sub-tropical floras are much richer in alkaloid-bearing species than the colder plant populations.

On no occasion did a ++++ or +++ precipitate result and one must be a little critical of lighter quantities of precipitate for impurities can interfere with the spot tests. The presence of bases in species yielding only slight precipitates should be confirmed by a more rigorous method e.g. the direct estimation of alkaloid by titration with toluene sulphonic acid (40, 41).

It should also be mentioned that not all alkaloids respond to the reagents used in this investigation. No single reagent has yet been developed to cover the whole range of natural bases. In addition, in some genera (e.g. Senecio) the bases occur in the plants

in the form of their amine oxides and as such do not give positive results with Meyer's solution. To overcome this difficulty reduction of the acid extract of the plant with nascent hydrogen prior to the addition of Meyer's solution was tried. However this did not alter the result of the test.

TABLE A.1

Legend

(X) Endemic in Tasmania

A. 1% Hydrochloric Acid

Pr. Prollius Solution

A.R. 1% Hydrochloric Acid extraction followed by reduction with zinc and acid

M. Meyer's Solution (38)

P. Saturated Aqueous Picric Acid

I. Potassium Iodide/Iodine Solution

Neg. No precipitate

\+ Indicates positive reaction ; + slight turbidity ; ++ heavier precipitate +++ very heavy precipitate ; etc.

PLANT	TIME & LOCATION OF COLLECTION	SOLVENT	RESULT
APOCYANACEAE			
Lyonsia straminia	January ; N.W. Coast	A	M+ I+
		Pr.	M I Neg.

PLANT	TIME & LOCATION OF COLLECTION	SOLVENT	RESULT
ARALIACEAE			
Panax gunii (X)	November ; Corinna, W. Coast	A	M P I Neg.
COMPOSITAE			
Bedfordia salicina	December ; Longley, Huon	A	M P I Neg.
Cassinia aculeata	December ; Longley, Huon	A	M P I Neg.
	December ; Snug Plains	A	M P I Neg.
	March ; W. Coast	A	M P I Neg.
Gnaphalium collinum	December ; Longley, Huon	A	M P I Neg.
Olearia floribunda	December ; Longley, Huon	A	M P I Neg.
	December ; Snug Plains	A	M P I Neg.
Olearia argophilla	November ; Corinna, W. Coast	A	M+ P+ I+

PLANT	TIME & LOCATION OF COLLECTION	SOLVENT	RESULT
COMPOSITAE cont'd			
Olearia gentisosa	November ; Corinna, W. Coast	A	M P I Neg.
Olearia glandulosa	March ; W. Coast	A	M P I Neg.
	January ; N.W. Coast	A	M I Neg.
		Pr	M I Neg.
		A.R.	M I Neg.
Olearia persoonoides (X)	December ; Snug Plains	A	M+ P+ I+
Olearia ramulosa	November ; Corinna, W. Coast	A	M P I Neg.
Olearia stellulata	March ; W. Coast	A	M P I Neg.
	November ; Corinna, W. Coast	A	M P I Neg.
	December ; Longley, Huon	A	M P I Neg.
Olearia viscosa	December ; Longley, Huon	A	M P I Neg.

PLANT	TIME & LOCATION OF COLLECTION	SOLVENT	RESULT
COMPOSITAE			
Ozothamnus hookeri	March ; W. Coast	A	M P I Neg.
	November ; Corinna, W. Coast	A	M P I Neg.
Senecio australis	March ; W. Coast	A	M P I Neg.
	November ; Corinna, W. Coast	A	M P I Neg.
	December ; Snug Plains	A	M P I Neg.
Senecio centropappus (X)	January ; N.W. Coast	A	M I Neg.
		Pr	M I Neg.
		A.R.	M I Neg.
Senecio dryadeus	December ; Longley, Huon	A	M+ P+ I+
Senecio lautus	December ; Longley, Huon	A	M+ P I Neg.
Senecio villeyoides	November ; Corinna, W. Coast	A	M++ P++ I++

PLANT	TIME & LOCATION OF COLLECTION	SOLVENT	RESULT
Senecio villeyoides cont'd	January, N.W. Coast	A Pr. A.R.	M+ I+ M I Neg. M I Neg.
CRUCIFERAE Cardamine stylosa	December ; Snug Plains	A	M++ P++ I++
DILLENIACEAE Hibbartia ovata	December ; Snug Plains	A	M P I Neg.
DROSERACEAE Drosera binata	December ; Snug Plains	A	M P I Neg.
EPACRIDACEAE Cyathodes ascendens (X)	November ; Corinna, W. Coast	A	M P Neg. I+

PLANT	TIME & LOCATION OF COLLECTION	SOLVENT	RESULT
ERICACEAE			
Gaultheria hispida	November ; Corinna, W. Coast	A	M P I Neg.
LABIATAE			
Prostanthera lasianthos	November ; Corinna, W. Coast	A	M P I Neg.
	December ; Snug Plains	A	M+ P I Neg.
Westringia rigida	December ; Longley, Huon	A	M P I Neg.
	December ; Snug Plains	A	M P I Neg.
LEGUMINOSAE			
Acacia melanoxylon	November ; Corinna, W. Coast	A	M P I Neg.
Acacia microcarpa	March ; W. Coast	A	M P I Neg.
Acacia riceana (X)	December ; Longley, Huon	A	M P I Neg.
	December ; Snug Plains	A	M P I Neg.

PLANT	TIME & LOCATION OF COLLECTION	SOLVENT	RESULT
LEGUMINOSAE cont'd			
Oxylobium ellipticum	December ; W. Coast	A	M P I Neg.
	December ; Snug Plains	A	M P I Neg.
Pultanea juniperina	December ; Snug Plains	A	M P I Neg.
LILIACEAE			
Blandfordia sp. (X)	March ; W. Coast	A	M P I Neg.
Milligania sp.	March ; W. Coast	A	M P I Neg.
MAGNOLIACEAE			
Drimys aromatica	November ; Corinna, W. Coast	A	M P I Neg.
OLEACEAE			
Notelaca ligustrina	December ; Snug Plains	A	M+ P I Neg.

PLANT	TIME & LOCATION OF COLLECTION	SOLVENT	RESULT
PERNETTYA			
Pernettya tasmanica (X)	July ; National Park	A	M P I Neg.
PITTOSPORACEAE			
Pittosporum bicolor	November ; Corinna, W. Coast	A	M P I Neg.
PROTEACEAE			
Banksia marginata	December ; Snug Plains	A	M P I Neg.
Cenarrhares nitida (X)	November ; Corinna, W. Coast	A	M P I Neg.
Hakia microcarpa	March ; W. Coast	A	M P I Neg.
Lometia polymorpha (X)	March ; W. Coast	A	M P I Neg.
Lometia tinctoria (X)	November ; Corinna, W. Coast	A	M P I Neg.

PLANT	TIME & LOCATION OF COLLECTION	SOLVENT	RESULT
RUBIACEAE			
Coprosma billerdiera	November ; Corinna, W. Coast	A	M P I Neg.
	December ; Longley, Huon	A	M P I Neg.
Coprosma nirtella	November ; Corinna, W. Coast	A	M++ P++ I++
	December ; Snug Plains	A	M P I Neg.
RUTACEAE			
Acradenia franklinii (X)	November ; Corinna, W. Coast	A	M P I Neg.
Correa laurineiana	December ; Longley, Huon	A	M P I Neg.
	December ; Snug Plains	A	M P I Neg.
Eriostemon squamus	March ; W. Coast	A	M P I Neg.
	November ; Corinna W. Coast	A	M+ P+ I+

PLANT	TIME & LOCATION OF COLLECTION	SOLVENT	RESULT
RUTACEAE cont'd			
Phlebalum squammeus	December ; Snug Plains	A	M+ P I Neg.
Zieria smithii	November ; Corinna, W. Coast	A	M P I Neg.
SAXIFRAGACEAE			
Anodopetalum biglandulosum	November ; Corinna, W. Coast	A	M P I Neg.
Anopterus glandulosus (X)	November ; Corinna, W. Coast	A	M P I Neg.
Baura ruboides	December ; Snug Plains	A	M P I Neg.
Eucryphia billardiera (X)	November ; Corinna, W. Coast	A	M P I Neg.
SCROPHULARIACEAE			
Euphrasia brownii	March ; W. Coast	A	M P I Neg.

PLANT	TIME & LOCATION OF COLLECTION	SOLVENT	RESULT
SCROPHULARIACEAE cont'd			
Euphrasia sp.	November ; Corinna, W. Coast	A	M P I Neg.
SOLANACEAE			
Solanum nigrum	March ; Hobart	A	M I Neg.
THYMELIACEAE			
Pimelia ligustina	November ; Corinna, W. Coast	A	M P I Neg
Pimelia linifolia	November ; Corinna, W. Coast	A	M P I Neg.
Pimelia pigmea (X)	November ; Corinna, W. Coast	A	M P I Neg.

PLANT	TIME & LOCATION OF COLLECTION	SOLVENT	RESULT
TILIACEAE			
Aristotelia peduncularis (X)	March ; W. Coast	A	M+ P+ I+
	November ; Corinna, W. Coast	A	M P I Neg.
	December ; Snug Plains	A	M P I Neg.

APPENDIX B

INVESTIGATION OF THE BARK OF TAKINI

In the Dutch territory of Surinam grows a tree which the natives of that part call Takini. Its botanical classification is obscure although in all probability Takini belongs to one of the genera Piratinera or Brosimum which are members of the family Moraceae (67). The natives of Surinam tap the sap from the Takini tree and from this liquid they make a beverage which produces strange hallucinations. The partaking of such a potion apparently has some religious significance as the natives claim that during the ensuing dreams contact is made with the "Takini gods". A similar preparation is made from a Nicotania sp. which enables contact with the "Tobacco gods".

A quantity of Takini bark was made available to the present writer so that some effort might be made to discover the physiologically active agent responsible for the hallucinations. As a preliminary purification the ground bark was extracted with benzene in a Soxhlet type apparatus. Since the native concoction arises from the sap of the tree it appears most likely that the active compound was water

soluble and would be left undissolved by the benzene. Subsequently a further methanol extraction over an extended period of time was carried out to remove this compound if indeed such was present in the dried bark. The present report deals with some crystalline compounds isolated from a benzene solution ; details of the methanol extraction will be given at some future date.

By means of a chromatographic technique, the details of which appear in the following experimental part, eight crystalline compounds (designated for convenience A-H) were isolated from the benzene extract of the Takini bark. However some of these were obtained in such small amounts that a detailed examination was impossible.

The properties and physical constants of compound C (0.01%) closely resembled (see Table B.1) those reported in the literature (65, 66) for the " cork alcohol ", friedelin. By the kindness of Professor Ruzicka a sample of this compound was made available for direct comparison with the material isolated from Takini. No melting point depression was noted when the two samples were heated together. Hence it appears that friedelin occurs in the bark of Takini.

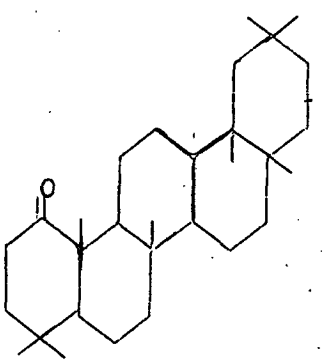
TABLE B.1

	Compound C	Friedelin (65, 66)
m.p.	256°	255-61°
$[\alpha]_D$ ($CHCl_3$)	-19	-29
Oxime m.p.	292-5°d.	290-4°d.

Although friedelin has been known for a number of years it is only quite recently that a complete structure has been proposed for this compound. Drake and his collaborators (65, 66, 103, 108, 109, 110) and later Ruzicka and Jeger (68, 102) have shown that friedelin is a saturated pentacyclic ketone, $C_{30}H_{50}O$, and that the immediate environment of the carbonyl group in friedelin is portrayed by

$$-CH_2-CH_2-CO-CH-CH-$$

Spring and his co-workers in a very recent publication (111) have reported the complete structure of friedelin as shown below.



Compound F (0.007%), C_{25} H_{44} O (m.p. 194-7° $[\alpha]_D$ = +26) showed a similarity in physical constants to an alcohol (C_{25} H_{44} O m.p. 193-4°, $[\alpha]_D$ = +16) obtained by Ruzicka and his co-workers (68) as a degradation product of friedelin. Professor Ruzicka and his colleagues have kindly compared these two compounds but report that they are not identical.

Compound E (C_{25} H_{50-52} O m.p. 81°) resembled a long chain alcohol, ceryl alcohol C_{25} H_{52} O m.p. 78°, a waxy material known to be present in plant material. The " frosting " of the leaves of a local Eucalyptus species (E. tasmanica) was shown by Bick and Komzak (69) to be a long chain alcohol (C_{25} H_{52} O, m.p. 79°), probably identical with ceryl alcohol. A mixed melting point determination of the alcohols from Takini and E. tasmanica showed a depression of about 3°. This indicates that the two compounds are not identical but may be closely related.

Compound A (C_{15} H_{26-28} O, m.p. 137°, $[\alpha]_D$ = -24, acetate m.p. 115°) is probably a new sesquiterpene alcohol. The name takinol is proposed for it.

Compound H (C_{22} H_{44} O_2, m.p. 84-6°) is isomeric with behenic acid (m.p. 81°) which is known to occur

in some waxes. However the former appeared to possess no acidic properties a fact which refutes the identity.

Compounds B (C_{25} H_{42} O, m.p. 274-6°), D (m.p. 118-9°), G (m.p. 75°) were present in such small amounts that little information could be gained about them.

EXPERIMENTAL

Dried and ground Takini bark (2700 g.) was extracted in a Soxhlet with benzene for a period of three days. The benzene solution was concentrated *in vacuo* to 500c.c., filtered to remove a little suspended matter and the clear filtrate chromatographed on neutral alumina (300 g.). The column was eluted with the following solvents.

Benzene	eluate A
50% Benzene ... 50% Chloroform	eluate B
Chloroform	eluate C

Eluate C. Chloroform washed the major part (7 g.) of the absorbed material from the column but when the solvent was removed a waxy semi-solid material remained which refused to crystallise from the usual solvents. This was set aside for future examination.

Eluate B. Benzene-chloroform (1:1) eluted a colourless crystalline compound (0.2 g.) which was purified by recrystallisation from petroleum ether (40-60°) when it had m.p. 137° and $[\alpha]_D^{19} = -24$ (c, 0.3 in $CHCl_3$ in 4 dcm. tube). This was designated as compound A.

Found

C,80.6 H,12.15 O,7.0 %

Calc. for $C_{15}H_{26}O$

C,81.0 H,11.8 O,7.2%

Calc. for $C_{15}H_{28}O$

C,80.3 H,12.6 O,7.1 %

Benzene-chloroform also produced a bright orange eluate easily separable from compound A on the column as a result of its colour. However this fraction refused to crystallise.

Compound A Acetate (Takinol Acetate). Compound A (0.08 g.) was mixed with pyridine (1 c.c.) and acetic anhydride (1 c.c.) and the whole refluxed for one hour. After cooling, the mixture was added to ice cold water (5 c.c.) and the precipitated material removed by filtration. After washing with hydrochloric acid (1%) and water the compound was crystallised from ethanol when it had m.p. 115°.

Eluate A. Benzene washed solid waxy material (2.0 g.) from the column ; this was redissolved in petroleum ether (40-60° ; 500 c.c.) and rechromatographed on alumina. Elution with the undermentioned solvents

followed using an automatic fraction-collector.

Petroleum Ether (40-60°)	eluate D
50% Petroleum Ether (40-60°) 50% Cyclohexane	eluate E
Cyclohexane	no material removed
50% Cyclohexane .. 50% Benzene	eluate F
Benzene	no material removed

Eluate D. Removal of the solvent left a crystalline material which was further purified by recrystallisation from petroleum ether (40-60°). At this stage this compound, called compound B, was obtained as colourless plates m.p. 274-6°.

Found C,84.0 H,11.5%

Calc. for $C_{25}H_{40}O$ C,84.2 H,11.3%

Calc. for $C_{25}H_{42}O$ C,83.7 H,11.8%

The mother liquors from the recrystallisation of compound B yielded a very small amount of yellow cream needles, m.p. 118-9° (compound D).

Eluate E. Petroleum ether-cyclohexane (1:1) removed a small amount of crystalline material (0.05 g. ; compound H) which was recrystallised from petroleum ether when it had m.p. 84-6°.

Found

C,77.5 H,12.9%

Calc. for $C_{22}H_{44}O_2$

C,77.5 H,13.0%

Eluate F. By the aid of an automatic fraction-collector, which collected 12 c.c. fractions, the washings from the cyclohexane-benzene (1:1) elution could be separated into four compounds. Thirty fractions were collected in all.

Tubes	1-11	Compound C	m.p. 254°
	12-15	Compound E	m.p. 80°
	16-22	Compound F	m.p. 180-5°
	23-30	Compound G	m.p. 70°

Compound C (Friedelin). Recrystallisation of compound C from petroleum ether (40-60°) yielded fine needles (0.3 g.), m.p. 256° and $[\alpha]_D^{19} = -19$ (c, 0.3 in $CHCl_3$ in 4 dcm. tube). Duke's carbonyl reagent (71) gave a positive test when compound C was subjected to it for an extended period.

Found

C,84.3 H,11.8 O,3.7% M.Wt.,485 (Rast)

Calc. for $C_{30}H_{50}O$

C,84.4 H,11.8 O,3.7% M.Wt.,426

Drake et al. (65, 66) report 255-61° and $[\alpha]_D$ = -29 for friedelin. The m.p. of compound C was undepressed by an authentic sample of friedelin.

Oxime of Compound C - Friedelin Oxime. Compound C (0.1 g.) was dissolved in benzene-alcohol (20 c.c. : 0.5 c.c.) and hydroxylamine hydrochloride (0.35 g.) dissolved in alcohol (2.5 c.c.) was added followed by alcoholic potassium hydroxide (0.3 g. in 2.5 c.c.). The mixture was refluxed for one hour, cooled and poured into water (40 c.c.). The resultant solution was acidified with sulphuric acid (10%) and the product filtered, washed and recrystallised from benzene-ethyl acetate (2:1) when the oxime had m.p. 292-5°d. Drake et al. (66) indicate 290-4°d. for the m.p. of friedelin oxime.

Compound E. Recrystallisation of compound E (0.05 g.) from petroleum ether (40-60°) gave needles m.p. 81°.

Found

C,82.0 H,13.6% M.Wt.,345 (Rast)

Calc. for $C_{25}H_{50}O$

C,81.9 H,13.7% M.Wt.,366

Calc. for $C_{25}H_{52}O$

C,81.7 H,14.2% M.Wt.,368

Compound F. Compound F (0.2 g.) was recrystallised from petroleum ether (40-60°) and ethanol to give colourless needles m.p. 194-7° (sintering 185°), $[\alpha]_D^{19} = +26$ (c, 0.3 in $CHCl_3$ in 4 dcm. tube).

Found

C,83.1 H,12.1 O,4.5% M.Wt.,342 (Rast)

Calc. for $C_{25}H_{44}O$

C,83.2 H,12.3 O,4.4% M.Wt.,360

Compound G. Compound G was recrystallised from petroleum ether (40-60°) to give a minute amount (ca 0.01 g.) of colourless needles m.p. 75°.

SUMMARY

Atherospermine, the major base of *Atherosperma moschatum*, has been shown to be identical with berbamine and the phenolic group in this base has been placed unequivocally.

Sodium in liquid ammonia reductive cleavages have been used to investigate structural problems which could not be resolved by standard degradative methods. In particular the secondary nitrogen group has been placed in daphnoline and daphnandrine while more information has been gained concerning the placement of hydroxyl groups in aromoline, daphnandrine, daphnoline, *iso*-chondrodendrine and chondrocurine. Observations on reductive fission experiments on dauricine and repanduline have been recorded.

Some stereochemical problems associated with repandine and oxyacanthine have been discussed.

Extractions have been performed using modern techniques and these procedures have revealed the presence of new compounds in *Berberis vulgaris* (*iso*tetrandrine, berberine oxalamide) and *Chondrodendron tomentosum* (tomentosine, N-benzylphthalimide). Extractions on the *Daphnandras* have revealed further irregularities in their alkaloid content.

The results of a survey of some Tasmanian plants for alkaloid-bearing species have been tabulated.

Friedelin together with some other non-basic compounds have been isolated from the bark of Takini, a tree native to Surinam, South America.

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